

# BULLETIN OF THE RESEARCH COUNCIL OF ISRAEL

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# BULLETIN OF THE RESEARCH COUNCIL OF ISRAEL

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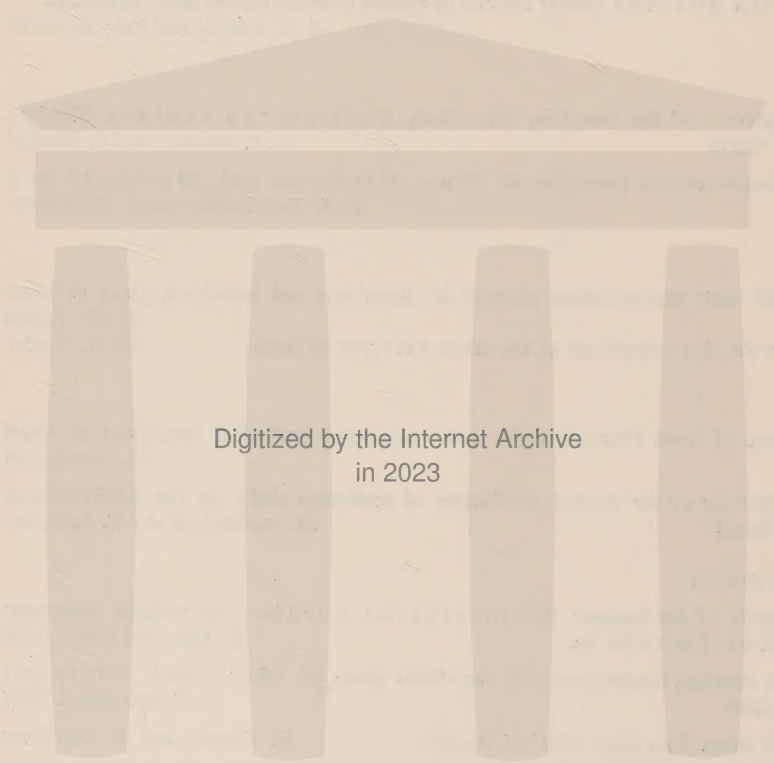
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# THE DIAGNOSIS OF SEX BEFORE BIRTH USING CELLS FROM THE AMNIOTIC FLUID (A PRELIMINARY REPORT)

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## ABSTRACT

It has been shown in humans, that cells from the amniotic fluid can be used to diagnose the sex of the foetus before birth.

In humans, as in most other mammals, males normally have the sex chromosome constitution XY (references in Sachs 1954)) and females the sex chromosome constitution XX. In sexually normal individuals, a determination of the sex chromosome constitution therefore gives a diagnosis of sex.

Such a determination can be most readily made on the basis of the percent of cells with chromocentres, especially those at the nuclear membrane, since this has been shown to give a clear sex difference in a variety of tissues in humans and some other species (Emery and McMillan 1954, Graham 1954, Marberger and Nelson 1955, Marberger et al. 1955, Moore and Barr 1954, 1955, Moore et al. 1953, Sachs and Danon, *Lancet* 1955). It thus seemed possible to us that this difference in the percent of cells with chromocentres could be used for determining the sex of viable human embryos before birth by using cells from the amniotic fluid.

It was first necessary to establish that cells suitable for the diagnosis are present in the amniotic fluid. Fluid was taken by puncture of the membranes from women in the ninth month of pregnancy, prior to delivery. The fluid was centrifuged, and the cells smeared on slides, fixed in alcohol-ether, and stained with Feulgen and fast green.

An examination of twenty such cases has shown that the fluid contains suitable cells, and an analysis of these cells has in all cases given a correct diagnosis of the sex of the foetus. The cases used in this study were obtained through the courtesy of the clinic of Professor B. Zondek and other centres.

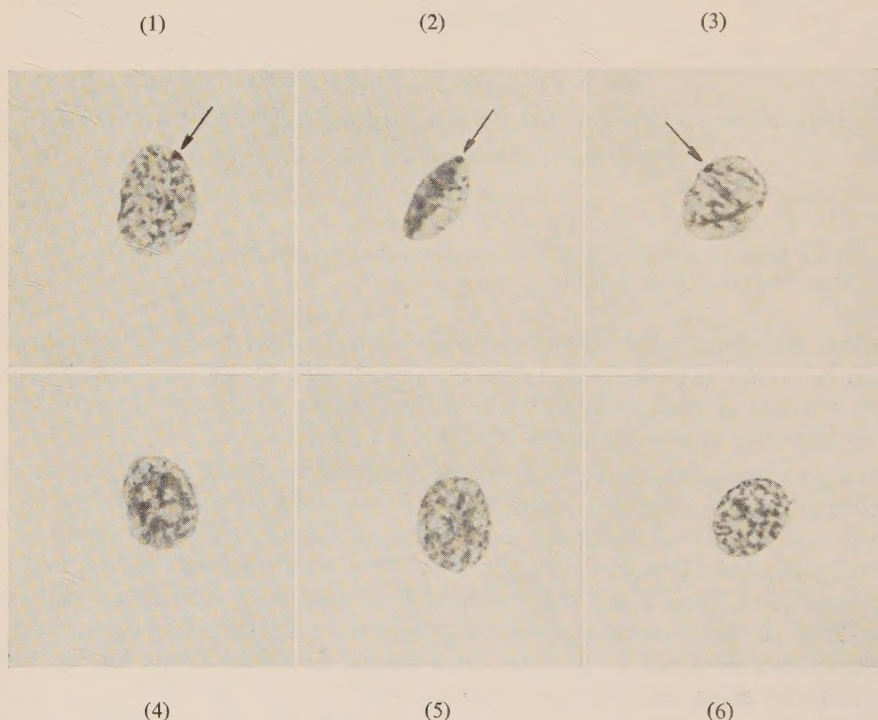
It seems that the only possible error in the present method of diagnosis, and this can be ignored for practical purposes, is in the rare case of an intersex in which the sexual phenotype appears to be in contradiction to the sex chromosome constitution. It is, of course, essential to avoid contamination with cells from the mother when collecting the amniotic fluid.

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Fluid can be obtained from viable human foetuses from twelve weeks to term (Alvarez and Caldeyro 1950, Dieckmann and Davies 1933), and we have already found that cells suitable for the diagnosis are present in fluid taken by abdominal puncture from a five and a half month old viable human foetus. A diagnosis could also be made from cells in the fluid of an eight week old aborted human foetus.

In extending this series, we are now collecting fluid, by various techniques, from viable human foetuses at other stages of pregnancy. It may also be possible to apply this method of diagnosing sex before birth to domestic animals.



Microphotographs of cells from the amniotic fluid. Figures 1, 2 and 3; nuclei with a chromocentre at the nuclear membrane, from female human foetuses in the ninth month. Figures 4, 5 and 6; nuclei without chromocentre, from male human foetuses in the ninth month.  $\times 1500$

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# THE EFFECT OF TRYPSIN ON LOCALIZED INFLAMMATION IN THE LIVER \*

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## ABSTRACT

- 1) The effect of trypsin on the organization of surgical gut in the liver of rats was investigated.
- 2) Intravenously administered trypsin caused marked depression of granulation tissue surrounding implanted surgical gut as well as marked fibrinolysis.
- 3) These effects did not occur when trypsin in oil was injected intramuscularly.

The antiphlogistic effect of trypsin has been reported both in clinical studies (Innerfield et al. 1953; Innerfield 1954, 1954a) and in experimentally induced edema (Martin et al. 1953, 1954; Beiler et al. 1955; Cohen et al. 1955; Adamkiewicz et al. 1955). The present report describes the effect of parenteral trypsin on the tissue response to implanted surgical gut in the liver.

## MATERIALS AND METHODS

Male albino rats bred at the Hebrew University weighing 200—300 g were maintained on Purina Laboratory chow, and tap water *ad libitum*. Details of the technique used and its critical evaluation have already been reported (Ungar and Neuman 1952, Ungar and Feldman 1953).

During laparotomy under ether anesthesia, plain surgical gut 3/0 ("Ethicon brand") was introduced into the main lobe of the liver using a straight needle.

The following preparations of trypsin were used:

- 1) Trypsin twice crystallized containing 50%  $\text{MgSO}_4$  (General Biochemical Inc.).
- 2) Trypsin 1 : 250 (Difco Laboratories).

These preparations were administered intravenously via a tail vein. All solutions were made up in phosphate saline buffer M/15 pH 7.4 and were freshly prepared daily.

3) Trypsin in oil (Parenzyme) obtained through the courtesy of the National Drug Company, Philadelphia. This was injected intramuscularly.

## *Determination of proteolytic activity*

To mixtures containing 0.2 ml rat plasma and various concentrations of trypsin, thrombin (Upjohn Co.) was added to a final concentration of 5 units per ml of mixture

\* This investigation was supported by the Hadassah Medical Organization research fund.

and the time taken for complete lysis of the clot was determined. Thus the dosage of a single injection was based on the minimal amount of trypsin needed for complete lysis of the normal rat plasma clot in 15 minutes at 37°. Blood volume was taken to be 10% of body weight.

The animals were examined on the fourth day following implantation of gut. Controls without trypsin were included in each experimental group. Slices of liver containing the gut were fixed in Zenker's acetic acid solution. Paraffin sections were stained with hematoxylin-eosin and by Laidlaw's silver impregnation counterstained with Van Gieson. For some animals Weigert's fibrin stain was used.

## RESULTS

### *Untreated controls*

The surgical gut was surrounded by abundant neutrophils mingled with remnants of necrotic liver cells. Around this area was a ring of granulation tissue 250—300  $\mu$  in thickness. The tissue contained moderate numbers of argyrophilic fibres, capillaries, young fibroblasts and histiocytes and was clearly defined against the adjacent liver tissue (Figure 1). These findings were identical with those reported previously (Ungar and Neuman 1952, Ungar and Feldman 1953). There was no mortality in this group and clot lysis was not observed.

### *Twice crystallized trypsin*

Of eight rats receiving trypsin, five survived to the end of the experiment, four days following the implantation of gut. Four to seven injections were given, starting 6—10 hours after the operation and continuing for 4 days. The total dose injected was 64—210 mg/kg body weight.

Blood coagulation time was considerably prolonged in all treated animals and ranged between 40 and 50 minutes as compared with 5 minutes in the controls. Marked fibrinolysis occurred in all animals, lysis of the clot being completed within 2—5 hours.

### *Histological findings*

The surgical gut was surrounded by neutrophil leukocytes which were present in diminished numbers in rats which had received more than 130 mg/kg of trypsin, as compared with those treated with the smaller dose. The ring of granulation tissue in all treated animals was narrower than in the controls and averaged 75—130  $\mu$  in thickness (limits 60—150  $\mu$ ). There were also signs of qualitative depression in the composition of granulation tissue. Fibroblasts appeared with dark nuclei, some of them tortuous, and were arranged in compact concentric rings. Mitosis was rarely seen. The numbers of histiocytes and capillaries were greatly reduced (Figure 2).

### *Trypsin 1 : 250*

Five of ten rats survived for four days. By that time they had received 360—640 mg/kg administered in 2—4 injections, starting 6—12 hours after laparotomy. Blood coagulation time ranged between 30 and 50 minutes throughout the experiment. Fibrinolysis tests gave essentially the same results as in the preceding group.



*Histological findings*

The gut was surrounded by a ring of granulation tissue 80--150  $\mu$  (limits 60--190) in thickness. The tissue contained less capillaries than in untreated animals but was rich in histiocytes. The nuclei of the fibroblasts were smaller and stained darker than in controls. Reticulum fibres were developed abundantly. The cellular elements of the granulation tissue were separated by an eosinophilic amorphous material which did not stain with Weigert's fibrin stain. Varying numbers of leukocytes were present in the inner zone of the reactive focus (Figure 3).

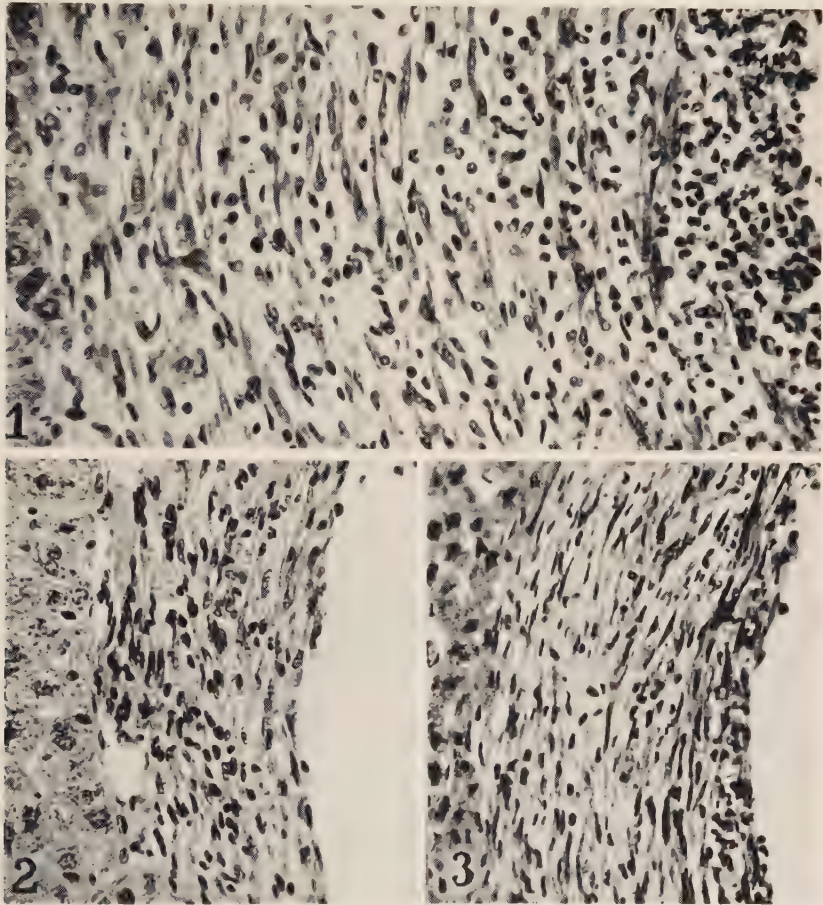


Figure 1  
Granulation tissue, four days after implantation of surgical gut into liver of normal rat. (At the right margin leukocytic exudate adjacent to the gut).  
Hematoxylin-eosin × 360.

Figure 2

Tissue response in liver following crystalline trypsin intravenously. Note absence of leukocytes and regression of granulation tissue.  
Hematoxylin-eosin × 360.

Figure 3

Tissue response in liver following crude trypsin intravenously. Changes are similar to preceding figure.  
Hematoxylin-eosin × 360.



*Intramuscular trypsin in oil*

During the 4 days of observation, 4 rats received a total of 13 mg/kg administered in 6 injections, and 4 others received 60 mg/kg administered in 5–6 injections. Injections were started 24 hours before laparotomy and the last one given 10 hours before death. Blood coagulation time remained normal as did prothrombin times and fibrinogen levels. No fibrinolysis could be detected, and all animals remained alive. The histological findings were essentially the same as in untreated controls; there was no visible suppression of granulation tissue.

## DISCUSSION

Experimental investigations of the effect of trypsin on inflammatory reactions have been mainly concerned with the acute stage of edema formation (Martin et al. 1953, 1954; Beiler et al. 1955; Cohen et al. 1955; Adamkiewicz et al. 1955). There is only one study which also deals with the effect of trypsin on chronic inflammation produced by kaolin, but no histological examination is reported (Adamkiewicz et al. 1955). No studies appear to have been made of the effect of trypsin on the granulation tissue response to absorbable foreign material. The implantation of plain surgical gut into the liver has previously been shown to be a useful method for the evaluation of factors concerning the development of fibroblastic tissue and its regression in the environment of the liver (Ungar and Neuman 1952, Ungar and Feldman 1953, Ungar 1953).

Trypsin, administered intravenously, was markedly toxic and nearly half of the animals died during the course of the experiment. No lethal effect was observed following the intramuscular injection of trypsin in oil.

The tissue response following intravenous injections of crystalline or crude trypsin was similar in all animals and differed from that in untreated controls in several respects. The ring of granulation tissue surrounding the surgical gut was significantly narrower, having an average thickness of 100  $\mu$  and being in no instance thicker than 190  $\mu$ , while in the control animals it measured 250–300  $\mu$ . The number of polymorphonuclear leukocytes and blood capillaries was reduced and fibroblast nuclei appeared deformed and frequently hyperchromatic. There was no interference with the formation of reticulum fibres. No fibrin was demonstrated in any case, by Weigert's stain.

Trypsin is known for its thromboplastic and proteolytic properties; in small doses it initiates blood coagulation while in high doses it is proteolytic (Innerfield et al. 1952). Trypsin is also known to be a potent activator of plasminogen, converting it to the proteolytic enzyme plasmin (Lewis and Ferguson 1952). In our experiments animals given intravenous crystalline trypsin showed high proteolytic activity of the blood, while trypsin in oil did not have this effect.

The histological findings do not establish whether the depressive effect of trypsin on granulation tissue formation is due to its proteolytic activity, lowering the plasma fibrinogen or otherwise affecting blood coagulation or by a direct interference with organization of the inflammatory exudate. The suggestion that the apparent anti-phlogistic action of trypsin in edema might be the result of hypersecretion of corticosteroids, secondary to stress, has not found experimental support (Adamkiewicz et al. 1955).

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# AN ATTEMPT TO PRODUCE A SPECIFIC SERUM AGAINST *PLASMODIUM BERGHEI* IN THE RABBIT

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There has been a small number of reports on the effect of serum taken from man and animals with previous attacks of malaria on the course of malaria infections in man and monkeys. Sotiriades (1917) used serum taken from a clinical case during a remission for the treatment of an acute malarial infection. His results are not definite because serum treatment was combined with quinine. Coggeshall and Cumm (1937) treated experimentally infected monkeys with serum of other monkeys taken during a latent phase. In these experiments, serum of susceptible animals in which some degree of immunity had been produced by previous attacks was used. We attempted to produce a plasmodicidal serum by repeated inoculation of blood heavily infected with *P. berghei* into a non-susceptible animal, i.e. the rabbit.

## METHODS

Two series of rabbits were used; one was subjected to repeated intravenous injections of heavily infected whole blood of hamsters, and the other to heavily infected mouse blood. The rabbits were given 6 intravenous injections at intervals of 4 days and were bled 7—10 days after completing the course. During the course of preparation, each rabbit received a total of ca.  $18.3 \times 10^9$  living parasites in the case of animals receiving infected mouse blood, and ca.  $16.1 \times 10^9$  living parasites in the case of hamster blood. For obvious reasons serum from rabbits prepared with infected mouse blood was used in experiments on hamsters and vice versa. Controls were treated with normal rabbit serum.

### *Survival of P. berghei in rabbits*

At various intervals after intravenous injections of hamster blood containing from 0.5 to  $2.5 \times 10^9$  parasites, blood was withdrawn from the rabbit and 0.5 ml injected into mice. Of 8 experiments with blood taken 5, 6, 18, 20 and 48 hours after intravenous injection, only one gave a positive result: a rabbit receiving its first intravenous injection ( $0.5 \times 10^9$  parasites) was bled 18 hours later and the blood was found infective for a mouse; two hours later and again 30 hours later, blood from the same rabbit gave negative results. It therefore appears that *P. berghei* disappears rapidly from the circulation



of rabbits subjected to intravenous injections containing relatively enormous numbers of parasites.

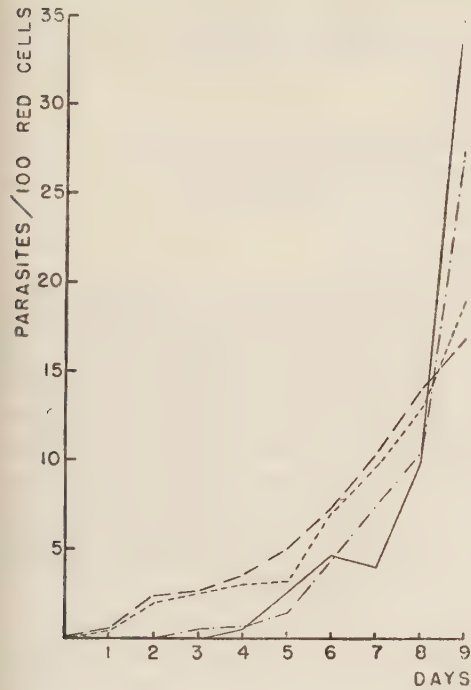


Figure 1  
Development of *P. berghei* in hamsters inoculated with  $40 \times 10^6$  parasites and treated with immune serum.  
— group of animals treated with 0.5 ml simultaneously with inoculation;  
- - - as above, simultaneously and daily;  
- . - . - . treated with 0.5 ml after appearance of parasites;  
..... group of animals without treatment—control.

*Effect of normal rabbit serum on the course of P. berghei infections in hamsters and mice*

The only significant effect of normal rabbit serum in doses of 0.5 ml is a slight prolongation of the incubation period in hamsters and mice. In 12 hamsters receiving 0.5 ml normal serum simultaneously with an inoculation of  $40 \times 10^6$  parasites, the average prepatent period was 3 days, in 35 control animals 2 days.

In 8 hamsters receiving 0.5 ml serum simultaneously with  $10^5$  parasites, the prepatent period was 4.5 days (in 2 instances, 6 days), in 8 controls the prepatent period was 4 days.

Daily injections did not prolong the incubation period. The intensity of infection in treated animals during the first 3 days of the prepatent period was distinctly slighter than in controls. Subsequently there was no significant difference as compared to controls.

In mice inoculated with  $10^5$  parasites, injections of 0.5 ml normal rabbit serum produced a prolongation of the prepatent period — an average of 3.2 days as against 1.3 days in controls. In mice inoculated with  $40 \times 10^6$  parasites, treatment with normal rabbit serum had no appreciable effect on the length of the prepatent period. The course of the infection after the prepatent period was not influenced by treatment with normal rabbit serum.

### *Effect of serum prepared by inoculation of infected mouse blood*

This serum had a very definite deleterious effect on hamsters; 6 animals, of which 2 received a single injection, 2 two daily injections, and one to three daily injections of 0.5 ml serum, died. All these animals had purpuric patches scattered over the whole abdomen. This phenomenon indicates the presence of antigens common to the hamster and the mouse.

The influence of the specific serum on the course of the infection in animals which survived for a sufficient period to warrant conclusions, was as follows.

### *Prophylactic and therapeutic*

Of 40 hamsters injected with serum simultaneously with the inoculation of parasites, 5 showed no infection during an observation period of 9 months. Of these 5 animals, 4 received an inoculation of  $40 \times 10^6$  parasites and simultaneously 0.5 ml serum; 3 of these animals received only a single injection of serum; one received daily injections of 0.5 ml serum for a period of 11 days. The fifth animal received an inoculation of  $10^5$  parasites simultaneously with 0.5 ml serum. This animal was treated daily with 0.5 ml serum for 8 days. It showed no parasites during an observation period of 9 months.

A sixth animal (1 out of 20), which was treated daily for 9 days immediately after the blood showed a small number of parasites, was apparently cured. It showed no parasites after the first injection during an observation period of 9 months.

In view of the fact that hamsters are invariably susceptible to *P. berghei* with an infection rate of 100% and a mortality approximating to 100%, the above results definitely show that specific serum prepared in the rabbit has a definite protective action against *P. berghei*.

### *Prepatent period in treated hamsters*

14 hamsters were inoculated with  $10^5$  parasites and simultaneously received an injection of 0.5 ml serum. They all became infected and the average incubation period was 4.2 days. 16 controls showed an average incubation period of 3 days. 6 hamsters received  $40 \times 10^6$  parasites and simultaneously 0.5 ml serum, and showed an average incubation period of 3.7 days. 25 controls showed an average incubation period of 2 days.

The specific serum produced a prolongation of the prepatent period approximately similar to but rather longer than that produced by normal serum.

### *Course of infection*

The course of infection in animals receiving a single injection of 0.5 ml serum simultaneously with the inoculation of parasites was not significantly different from that in animals receiving daily injections. During the first 3 days after the patent period the intensity of infection in treated animals was smaller than in controls. After this period the rate of multiplication in treated animals was significantly greater than in untreated controls. The survival in treated animals receiving daily injections of specific serum after an inoculation of  $10^5$  parasites (7 animals) was 8 days, in animals receiving a single injection 13 days and in (8) controls 16.2 days; in 9 animals receiving  $40 \times 10^6$

parasites and treated with a single injection the average survival was 12 days, in those receiving daily treatment (11 animals) the average survival period was 10.6 days, and 15.6 days in (12) untreated controls. (The discrepancy in the survival period in the two groups receiving a single injection of serum is due to the fact that animals which died with signs of purpura after a few days are included in the figures and their number was not equal in both groups).

We attribute the increase in multiplication rate after the fourth day of the patent period and the fall in survival period in treated animals to the deleterious effect of anti-mouse blood serum on the immune mechanism of hamsters which eventually (after 4 days) more than compensated for the protective action of the specific anti-plasmodium factor. In mice treated with specific serum the pre-patent period and course of infection were substantially the same as in animals treated with normal rabbit serum, i.e. there was a significant increase in the prepatent period, and a relatively lower intensity of infection during the first four days of patency. In no case was an infection prevented or cured. The course of the fulminating infection in the highly sensitive mouse was hardly influenced by specific serum prepared in rabbits from infected hamster blood.

The above findings can be summarized briefly. Specific serum prepared by intravenous injection of living parasites into rabbits has a definite effect on the course of *P. berghei* infections in hamsters. This is proved indubitably by the fact that in 5 animals infection was prevented by simultaneous injection of specific serum and parasites and in one animal treated at the commencement of the patent period the infection was eradicated. The intensity of infection in treated hamsters was significantly lower than in controls during the first three days of patency, but became subsequently greater and survival was briefer than in controls. This can be attributed to the pathological effect of anti-mouse serum in hamsters which occasionally succumb with signs of purpura.

Normal rabbit serum as well as specific serum prepared in rabbits prolong the prepatent period in mice and hamsters inoculated with *P. berghei*.

Specific serum is not significantly more effective than normal rabbit serum in mice infected with *P. berghei*. *P. berghei* was recovered from one rabbit on one occasion 18 hours after a massive injection of heavily infected mouse blood.

The action of specific serum, though distinct as evidenced by the prevention of infection in 5 hamsters out of 40 and eradication of a very slight infection in one hamster out of 20, is not to be compared to that of chemotherapeutic agents.

I wish to thank Professor S. Adler for suggesting the above subject for my M.Sc. thesis and for his kind advice.

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# ANTIMYCOTIC ACTIVITY OF STREPTOMYCETES ISOLATED FROM LOCAL SOIL \*

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## ABSTRACT

Out of 40 streptomycetes isolated from Ashkelon soil, 6 showed promising antifungal activity in primary screening tests against *C. albicans*. Three of these produced considerable activity in liquid media. Crude filtrates showed an activity of up to 500 *C. albicans* units per ml.

No antibiotics active against fungus infection in man have as yet been found, though several preparations have shown promising activity in vitro against various organisms causing superficial and deep mycoses.

Apart from Grisein (Reynolds et al. 1947) derived from a streptomycete isolated from Hula peat, no antibiotics have been recorded from Israeli soil. A systematic examination of streptomycetes from local soils was therefore undertaken.

A sample of soil collected in the vicinity of Ashkelon attracted attention because of its strong streptomycetic smell.

## MATERIALS AND METHODS

The soil was diluted with distilled water and plated in different dilutions with 10 ml of modified Czapek-Dox agar (I). Colonies of streptomycetes from plates containing 1 : 10<sup>5</sup> and 1 : 10<sup>6</sup> dilutions were transferred to Czapek-Dox agar slants and incubated at 26°C. Forty different strains of streptomycetes were isolated from this soil and tested for antifungal activity.

## Media

I. Czapek-Dox agar		II. Czapek-Dox broth	III. Glucose Agar	
NaNO <sub>3</sub>	0.2%	The same as I without agar.	Dextrose	2%
K <sub>2</sub> HPO <sub>4</sub>	0.1%		Peptone	1%
MgSO <sub>4</sub> · 7H <sub>2</sub> O	0.05%		Agar	1.5%
KCl	0.05%			
FeSO <sub>4</sub>	0.001%			
Dextrose	3%			
Soluble yeast extract (Difco)	0.2%			
Agar	1.5%			

\* This investigation was aided by a grant from the Hadassah Medical Organization.

### Primary screening

The streptomycetes grown on slants and showing full sporulation were streaked diametrically with a rectangularly bent needle heavily charged with spores on Czapek-Dox agar distributed in Petri dishes. After incubation at 26°C for three days, cross streaks of rich spore suspensions of the test organism were made and the plates again incubated. Three days later cross streaking was repeated on the other half of the plates. Clear zones between the edges of the streptomycetes colony and the test organism were measured. The presence of such zones was assumed to indicate production of an antifungal principle.

### Secondary screening

In an attempt to isolate active principles indicated in the primary screening test, the streptomycetes were grown in Czapek-Dox broth (II). The medium was dispensed in 100 ml quantities in 250 ml Erlenmeyer flasks and autoclaved at 15 atm. for 15 minutes. A well sporulated slant of the organism was flooded with sterile saline, the spores scraped off, and 1 ml of the resulting spore suspension inoculated into each flask. The flasks were kept at about 28°C in a Ross-Kershaw continuous shaking machine at 148 strokes per minute. After three, five and eventually seven days of shaking, 10–15 ml of the broth were withdrawn aseptically and paper-filtered. The pH of the clear filtrate was determined by a Beckmann glass electrode pH-meter. The crude filtrate was then heated at 60°C for 10 min and assayed by the agar dilution streak method. The activity of the crude filtrate was expressed as dilution units (*u*). (Dilution units indicate the highest dilution of 1 ml crude filtrate in agar completely suppressing the growth of the test organism). The test organism in both screening procedures was *C. albicans* freshly isolated from lesions of moniliasis of the skin.

### RESULTS

Of 40 soil streptomycetes tested by primary screening, only 6 showed appreciable activity against *C. albicans* (Table I). Table II shows the results of the secondary screening test on liquid medium. The filtrates of only 3 organisms out of the 6 active on primary screening showed antifungal activity. The activity increased with incubation time and roughly paralleled the rise of the pH of the filtrates. After the 6th or 7th day of growth the activity dropped sharply. By this time a considerable amount of lysis of the organism had occurred in most cultures (younger cultures consist of small well defined pellets). Extracts containing the active principle in *n*-butanol could be prepared from the crude filtrates of the three organisms\*.

### COMMENT

The primary screening test is relatively simple and crude, but may give useful practical indications. If cross streaks are made on the same plate at different times the size of the inhibition zones would at least indicate the rate of diffusion although this does not necessarily indicate the amount of antibiotic produced. From Table II it can be seen that relatively active filtrates with a titre up to 500 *C. albicans* units were obtained in liquid medium subjected to continuous shaking.

\* Work on all three organisms is being continued. From organism No. 33 a highly active, partly purified, relatively non-toxic solid has been isolated. This work will be recorded later.

TABLE I

Primary screening by cross streak technique on Czapek-Dox agar. Test organisms: *C. albicans*. Results read 48 hrs after cross streaks. Incubation at 26°C.

Streptomyces strain No.	mm of inhibition of <i>C. albicans</i> after 3 days growth of streptomycetes	mm of inhibition of <i>C. albicans</i> after 6 days growth of streptomycetes
3	16	12
4	15	23
12	16	20
16	16	0
33	12	0
34	24	0

TABLE II

Activity of crude filtrates of streptomycetes expressed in *C. albicans* units. Agar dilution streak technique. Results read after incubation at 26°C for 48 hrs.

Streptomycetes strain No.		3		4		12		16		33		34	
Days of growth on liquid medium		pH	u/ml	pH	u/ml	pH	u/ml	pH	u/ml	pH	u/ml	pH	u/ml
3		8.57	0	8.0	250	7.95	400	8.2	250	8.3	40	7.88	800
5		—	—	8.15	500	8.1	500	8.57	50	8.35	400	8.35	400
7		—	—	8.4	0	8.32	40	—	—	8.35	80	8.65	0

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# ATTEMPTS TO OBTAIN NON-DEPENDENT REVERTS FROM A STREPTOMYCIN-DEPENDENT MUTANT OF *BRUCELLA ABORTUS* (STRAIN 19) AND TO REPLACE STREPTOMYCIN BY OTHER SUBSTANCES\*

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## ABSTRACT

The experiments reported prove the rigid specificity of the dependence of the D-strain of *B. abortus* on streptomycin. Various organ extracts, neomycin and streptomycin, were not able to replace streptomycin. This result is interesting in view of the findings of Pagano, Weinstein and Donovan (1952), who reported a cross-resistance in a number of organisms to streptomycin, neomycin and streptomycin. On solid trypticase soya media with or without streptomycin, inositol or glucosamine, the D-strain grew in 1—2 subcultures. The strain never attained visible growth in the third subculture but grew again when transferred back to streptomycin medium.

It is known that in populations of streptomycin-dependent mutants non-dependent revertants may appear. Herzberg and Elberg (1953) revealed among  $10^8$  streptomycin-dependent microorganisms of *Brucella melitensis* 3 — 6 non-dependent revertants. On the other hand, Olitzki and Szenberg (1953) did not reveal any non-dependent mutants in a culture of *B. abortus* (strain 19).

Elberg and Herzberg (1955) showed that guinea pigs, which are highly sensitive to *Brucella* infections, are not infected by 600 non-dependent revertants injected together with  $10^{10}$  dependent *B. melitensis*. However, since living streptomycin-dependent *Brucellae* were used in immunization experiments (Elberg and Herzberg 1955, Herzberg and Elberg 1953, Herzberg, Elberg and Meyer 1953, Olitzki 1952, Olitzki and Szenberg 1953), it seemed important to examine whether body fluids from organs of different laboratory animals contain any substances which may replace streptomycin or stimulate the reversion to non-dependent virulent organisms.

## *Attempts to obtain revertants in the presence of different organ suspensions*

The sensitivity of our methods was examined as follows:

The *Brucellae* were grown in Roux bottles containing trypticase soy agar (Baltimore Biological Laboratory) with 3% glycerol and 0.01% thiamine. The growth was washed off and distributed in Erlenmeyer flasks containing 100 ml trypticase soy broth. The most probable number method (Olitzki 1952, Olitzki and Szenberg 1953) was employed in order to determine the number of non-dependent revertants. In three groups of 5 flasks containing  $10^{11}$ ,  $10^{10}$ , and  $10^9$  *Brucellae* in 100 ml broth, without streptomycin no growth occurred; this result indicates that a population of  $10^{12}$  microorganisms was free of revertants.

\* Supported by Mr. Ben May, Mobile, Alabama, U.S.A.

In the next experiment, the sensitivity of this test to reveal the appearance of non-dependent (ND) amidst a population of dependent (D) microorganisms was tested. Each of ten flasks containing 30 ml broth received an inoculum of  $5 \times 10^{10}$  *D-Brucellae*; five of them received in addition 10 ND-*Brucellae*. The ten ND-*Brucellae* multiplied to visible growth in 3 out of 5 flasks within 14 days. No growth was observed in the 5 flasks which contained only D-bacteria. In 3 out of 5 flasks with an inoculum of  $5 \times 10^{11}$  D- and 10 ND-*Brucellae* growth of ND-bacteria appeared within 10 days.

We examined the influence of organ suspensions of the heart, muscle, liver, spleen, lungs, pancreas, ovary, testes and kidney in order to determine whether they contain a substance able to provoke the appearance of ND- in populations of D-microorganisms. The organs obtained from rabbits, guinea pigs and rats were thoroughly minced in mortars with glass powder and diluted in saline up to 1 g/100 ml. Two ml of this suspension were added to 8 ml broth in series of 5 test tubes inoculated with  $10^7$ ,  $10^6$  and  $10^5$  thrice-washed D-*Brucellae*.

The viability of the D-*Brucellae* was tested after incubation periods of 7 and 14 days by inoculation of 0.1 ml streptomycin. Abundant growth occurred within 7 days in the presence of the antibiotic, while in its absence no growth occurred. None of the organ suspensions active or inactivated by heating at 55°C for 2 hours were able to provoke the appearance of ND-reverts.

#### *Attempts to replace streptomycin by other antibiotics and organic substances*

Streptomycin was replaced by streptothricin, streptolin, neomycin, inositol, in the broth medium and inoculated with the D-strain. Five  $\mu\text{g/ml}$  streptolin permitted the growth of the streptomycin-sensitive and streptomycin-resistant parent strains, while 7.0  $\mu\text{g/ml}$  inhibited the growth; the D-mutant did not grow in concentrations of 0.5 — 20.0  $\mu\text{g/ml}$ . Neomycin permitted the growth of the streptomycin-sensitive parent strain in a concentration of 7.0  $\mu\text{g/ml}$  and inhibited it in a concentration of 10.0  $\mu\text{g/ml}$ . The respective figures for the streptomycin-resistant strain were 3.0 and 5.0  $\mu\text{g/ml}$  and for the dependent strain 0.5 and 1.0  $\mu\text{g/ml}$ .

100  $\mu\text{g/ml}$  streptothricin did not inhibit the growth of the streptomycin-sensitive and resistant parent strain nor of the D-strain. Other streptomycin-sensitive and resistant strains, e.g. *B. melitensis* and *B. suis*, showed the same resistance to streptothricin. A delayed growth was observed on streptothricin and inositol; subcultures were made on the surface of plain agar or agar which contained inositol or streptothricin. The results were as follows:

Primary broth culture ( $\mu\text{g/ml}$ )	Transferred to ( $\mu\text{g/ml}$ )	Growth
Streptomycin (100)	Plain agar	Ceased immediately
Inositol (10 and 100)	Inositol (10 and 100)	Ceased after 1 passage
	Plain agar	Ceased immediately
	Streptomycin (10 and 100)	Abundant
	Streptothricin (10 and 100)	Ceased immediately
Streptothricin (10 and 100)	Streptothricin (10 and 100)	Ceased after 1 passage

In another experiment the bacteria were grown on agar slants with or without the addition of 0.5% inositol or 0.5% glucosamine.

In the first subculture the D-strain grew abundantly in all media. Traces of growth appeared in the second subculture, and in the third subculture visible growths were not seen within an incubation period of 10 days. Inocula from the surface of the macroscopically sterile slant of the third subculture material produced abundant growth on streptomycin-agar, while no growths appeared when the same material was inoculated on the surface of plain agar.

### Conclusions

A temporary growth of a streptomycin-dependent *B. abortus* was observed on 1—2 subcultures on trypticase soya agar without streptomycin. In the third subculture the growth was finally arrested. The presence of different organ extracts, streptothricin, glycerol, inositol or glucosamine in medium did not change this result. Viable micro-organisms were still present in the third subculture, although macroscopic growth was not observed after an incubation of 2 weeks.

None of the above substances provoked the appearance of ND-reverts.

The antibiotics used in all the experiments described above were kindly provided by the Squibb Institute for Medical Research, New Brunswick, N.J.

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# THE OCCURRENCE OF THE AMERICAN BLUE CRAB, *CALLINECTES* *SAPIDUS* RATHBUN, IN ISRAEL WATERS

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## ABSTRACT

During a recent investigation of the Decapod fauna of the Mediterranean coast of Israel, the existence there of the American Portunid crab *Callinectes sapidus* Rathbun has been revealed. The species had not yet been reported from the Mediterranean but it has been found recently in European waters (the Atlantic coast of France, Holland and Denmark).

Its frequent occurrence and the presence of ovigerous females, are strong indications that *Callinectes* has been established in Israel waters. It is found in similar habitats as in eastern America, in river estuaries and further upstream in water of extremely low salinity.

The most probable suggestion as to how this species has been transported from the eastern coast of America to the Mediterranean is that small specimens of the crabs enter with the water into the ballast tanks of ships; they are thus transported in these tanks and released at the end of the voyage when the tanks are emptied.

During a recent investigation of the Decapod fauna of the Mediterranean coast of Israel, a juvenile female swimming-crab (cl. 13 mm, cb. 27 mm)\* from the mouth of the Heftsi-Bah river came to hand. The identification of this specimen caused some difficulties as it proved to belong to a species different from any known from the Mediterranean. The possibility that the specimen might be a juvenile of an Indo-West Pacific species, that had entered the Mediterranean through the Suez Canal, was then looked into, but with negative results. Finally, the fact that the species was collected in brackish water and that the American Portunid crab *Callinectes sapidus* Rathbun had been found recently in European waters, gave us a lead to the correct identity of the specimen, which proved to be a juvenile specimen of the American species. A direct comparison of our Israel specimen with specimens of *C. sapidus* of about equal size from Chincoteague and Sinepuxent bays, Maryland, U.S.A., which form part of the collections of the Rijksmuseum van Natuurlijke Historie at Leiden, definitely established its identity. A subsequent checking of the collections of the Sea Fisheries Research Station at Haifa revealed more specimens of this species. Among them were several adult males and ovigerous females, so that the presence of this species in Israel waters is now convincingly demonstrated.

\* cl. = carapace length, cb. = carapace breadth.

The following specimens have been examined :

(a) Mediterranean coast of Israel (exact locality and date of collecting unknown), 1 male, cl. 75 mm, cb. 175 mm; 1 female, cl. 58 mm, cb. 122 mm.

(b) Mouth of Heftsi-Bah river near Hadera, 21.XI.1951, 3 males, cl. 10 to 15 mm, cb. 20 to 30 mm; 1 female, cl. 13 mm, cb. 27 mm. One specimen of *Portunus pelagicus* (L.) was found in this lot (coll. E. Gottlieb).

(c) Mouth of Dalia river near Tantura, about halfway between Caesarea and 'Atlit, 26.X.1955, 3 males, cl. 80, 82, and 90 mm, cb. 190, 185, and 210 mm respectively. These specimens show a growth of Balanidae belonging to the species *Chelonibia patula* (Ranzani) (coll. A. Perlmutter).

(d) Haifa Bay, 28.X.1955, 2 ovigerous females, cl. 68 and 65 mm, cb. 160 and 145 mm respectively; a few other specimens were found in this lot. In this locality *Portunus pelagicus* (L.) is more frequently met with than *Callinectes* (coll. E. Gottlieb).

(e) Mouth of Na'aman river near Acre. Numerous specimens were found during the period from III to X.1955. No *Portunus* was observed here (coll. A. Perlmutter).

The large number of specimens caught, and the presence of ovigerous females, are strong indications that *Callinectes* is not just an incidental visitor of Israel waters, but actually has become established there. Our inquiries as to the possibility of *Callinectes* having been introduced on purpose, e.g., to start a crab fishery, showed this supposition to be false. The introduction of this crab evidently was entirely unintentional.

In Israel *Callinectes* is found in similar habitats as in eastern America. It is encountered most frequently in river estuaries, being sometimes found quite far up the rivers in water of extremely low salinity. In this respect *Callinectes* is quite different from the other large Portunid crab of the Israel coast, *Portunus pelagicus* (L.), a species of Indo-West Pacific origin which penetrated into the Mediterranean, and which is rather abundant in the sea off the Israel coast, being rarely met with in the mouths of rivers. Ovigerous females of *Callinectes* have been observed in Israel in the month of October, while juveniles were found in August, September, and October.

*Callinectes sapidus* Rathbun inhabits the east coast of America, where it is known from Nova Scotia to Uruguay. It is most abundant on the east and south coast of the United States between Massachusetts and Texas. In this region, and especially in the Chesapeake Bay area, the Blue Crab is of great economic importance, being the object of an intensive fishery which yields several millions of dollars a year.

There are very few previous records of *Callinectes sapidus* from the eastern Atlantic. The first of these was published by Bouvier (1901, p.16), who reported a large male specimen (cb.180 mm) which in the year 1900 was found alive in fresh water of the harbour of Rochefort, S.W. France. In 1951 Den Hartog and Holthuis reported four specimens of this species found in Holland. Two of these specimens may be passed over in silence, since they were boiled when found in 1950 on the North Sea shore of the S.W. part of the Netherlands and evidently had been thrown overboard from a ship after at first having been intended for human consumption. The other two specimens, both females (cb.135 and 153 mm), were captured alive in Dutch waters, the smaller female was taken in September 1932 in the Zaan river near Zaandam (N.W. of Amsterdam), the other specimen was collected in December 1934 in the Amsterdam harbour. Very soon after the publication of the paper of Den Hartog and Holthuis, in July 1951, a third specimen (cb. 120 mm) was found alive in Holland; it was taken in the Noordzeekanaal, the large shipping canal which connects Amsterdam with the North Sea. This find was first reported

upon by Wolff (1954, pp. 19, 20; 1954a, p. 188), who furthermore dealt with the capture in September 1951 of a female of this species (cb. 141 mm) in the Sound, N.E. of Copenhagen, Denmark; the Danish specimen was kept in captivity and after one moult attained a carapace breadth of 177 mm. To our knowledge no living specimens of *Callinectes*, other than the five above specimens from France, Holland, and Denmark, have been reported from the eastern Atlantic. It is therefore most unexpected that the species is as common and as widely distributed in Israel waters as our material shows it to be.

There have been several suggestions as to how this species and other crabs that have been found far from their homeland have been transported over the large distances that they covered. It does not seem likely that an active swimmer like *Callinectes sapidus* would have been transported by hiding among the growth of ships' bottoms. Also it seems improbable that these crabs reached the west coast of the Eurasian continent by "active swimming, presumably in the shadow of floating sea weeds or wood, tree trunks, etc., carried by the Gulf Stream" as Wolff (1954a) suggests; this certainly could not explain the presence of *Callinectes* in Israel, while it also seems unlikely for a species like *Callinectes sapidus*, which shows such a strong preference for brackish water. The third, and in our opinion the most acceptable solution is that small specimens of the crabs are taken in with the water with which the ballast tanks of ships are filled and that they are transported in these tanks; the crabs then are released in their new environment when at the end of the voyage the tanks are emptied. We fully agree with Wolff (1954, 1954a) that this probably is the way in which the crabs reached us.

The specimens collected in Israel waters (samples *c* and *e*) were kindly placed at our disposal by Dr. A. Perlmutter, of the State University of New York, U.S.O.M., who also provided us with his observations on the occurrence of these species at the mouth of rivers in Israel, during his temporary stay there as a fishery expert to the Israel Government.

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# DISCRIMINATIVE OPTICAL PERCEPTION OF *MUS*, *MICROTUS* AND *MERIONES* IN A MAZE

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## ABSTRACT

The comparison between the behaviour of white mice and two species of wild rodents of dominantly subterranean life habits (*Microtus guentheri* D.A. and *Meriones tristrami* Ths.) in a maze concerned with training to optical stimuli, gave the rather surprising results that both wild species are by no means inferior — and partly even somewhat superior — in their faculties to discern and to learn the differences of the experiment.

A series of experiments concerning the learning of optical stimuli by white mice in a maze was carried out by Miss Kornhauser under the direction of Prof. Wojtusiek at Krakow. This experience was used here to study the differences in the perception and in the learning of optical stimuli of the wild rodents *Microtus guntheri* D. A. and *Meriones tristrami* Ths. as compared to those of white mice, *Mus musculus*. The different species promised not only to reveal eventual differences between domesticated and wild rodents, but also to show whether the most strictly subterranean of the two wild species would show deficiencies and delay in learning the optical stimuli. To our surprise, this was not the case.

## METHOD

The maze employed was relatively simple (Figure 1). The animal entered a big chamber through swinging door A, then through another one B, opening both with its head. On the opposite side of the chamber were swinging door C leading to a corridor on the left, and swinging door D leading to a corridor on the right. On doors C and D were posted figures of a rectangle on one and a square on the other, or a large circle on one and a square on the other. The "good" door was the one with the smaller figure, and, if the animal entered it, it received a "reward" (food) and was allowed to rest there all day. If the animal chose the "bad" door, it was "punished" by finding itself in a corridor running around the chamber with its only exit, door B, leading back into the same chamber. Back in the chamber, the animal was again faced with the same choice as in the beginning of the experiment. The figures posted on doors C and D were

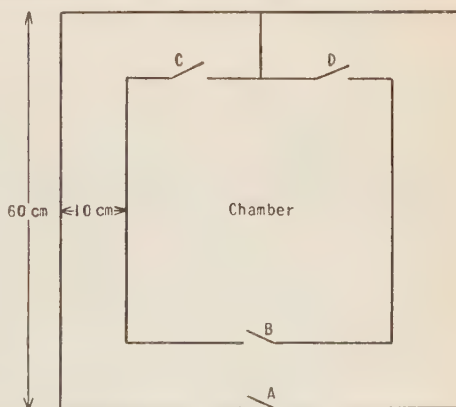


Figure 1  
Scheme of maze. A and B — entrance doors, C and D — choice doors.

alternated at intervals to assure that the animal did not learn to choose between the right and the left side nor get used to a rhythmic change.

After a number of positive responses the bigger figure was gradually reduced in size, the smaller one being left constant, until a limit was reached when the trained animal was no longer able to distinguish between the two figures and even very prolonged training gave no further results.

The mice were kept in separate cages on a diet of wheat, fruit and vegetables or milk. For 12 — 16 hours prior to an experiment, the wheat was omitted from the diet.

### RESULTS

All three species of rodents learned quite easily to distinguish between the figures. In one series of experiments, a square of 15 mm side length was contrasted with a rectangle of different side length which was progressively reduced (Figures 2 — 5). In a parallel series with *Microtus* only, a circle of a diameter of 10 mm was contrasted with circles of progressively decreasing diameters (Figure 6). The results showed learning in a maze by optical perception to be quite easy for all three species studied.

TABLE I  
*Speed of learning: the first day of experiments without error*

Square (15 mm side) vs. rectangle	<i>Mus</i>				<i>Microtus</i>		<i>Meriones</i>			Circle (10 mm diam.) vs. larger diam. (mm)	<i>Microtus</i>		
	A	B	C	D	A	B	A	B	C		C	D	E
30 × 15	16	17	17	15	24	25	23	22	18	20	12	13	9
28 × 15					9	13				18	7	5	5
27 × 15	15	13	13	10						16	5	7	5
25 × 15					7	7	6	6	4	14	3	7	5
24 × 15		10		9						12	+	7	+
22 × 15							8	8	6	11	=	=	=
21 × 15	7	7	7										
20 × 15					9	10	4	4	7				
18 × 15	+	7		5	5	5	4	4	4				
17 × 15	=	=		+	12	15	6	6	7				
16 × 15				=	=	=	=	=	=				

+ Number of errors still decreasing. = Number of errors not decreasing any more.

*Microtus* and *Meriones* learned more rapidly and perceived small differences in size better than *Mus*. *Microtus* remembered at least for several months shapes between which it had once learned to discriminate. It is noteworthy, however, that after the shock of an exhausting 6 hour journey from Haifa to Jerusalem *Microtus* showed a complete loss of memory in the first series of experiments three weeks later, but learned very rapidly in the following series (Figure 5). In a number of control experiments, when the second series was smaller than the standard shapes (square 15 × 15 mm or circle 10 mm diam.), all species turned to the smaller shapes after they had learned that this was the "good" solution.

These experiments show that, in spite of considerable ecological and ethological differences between the three rodent species studied, their capacity for optical discrimination was rather similar.

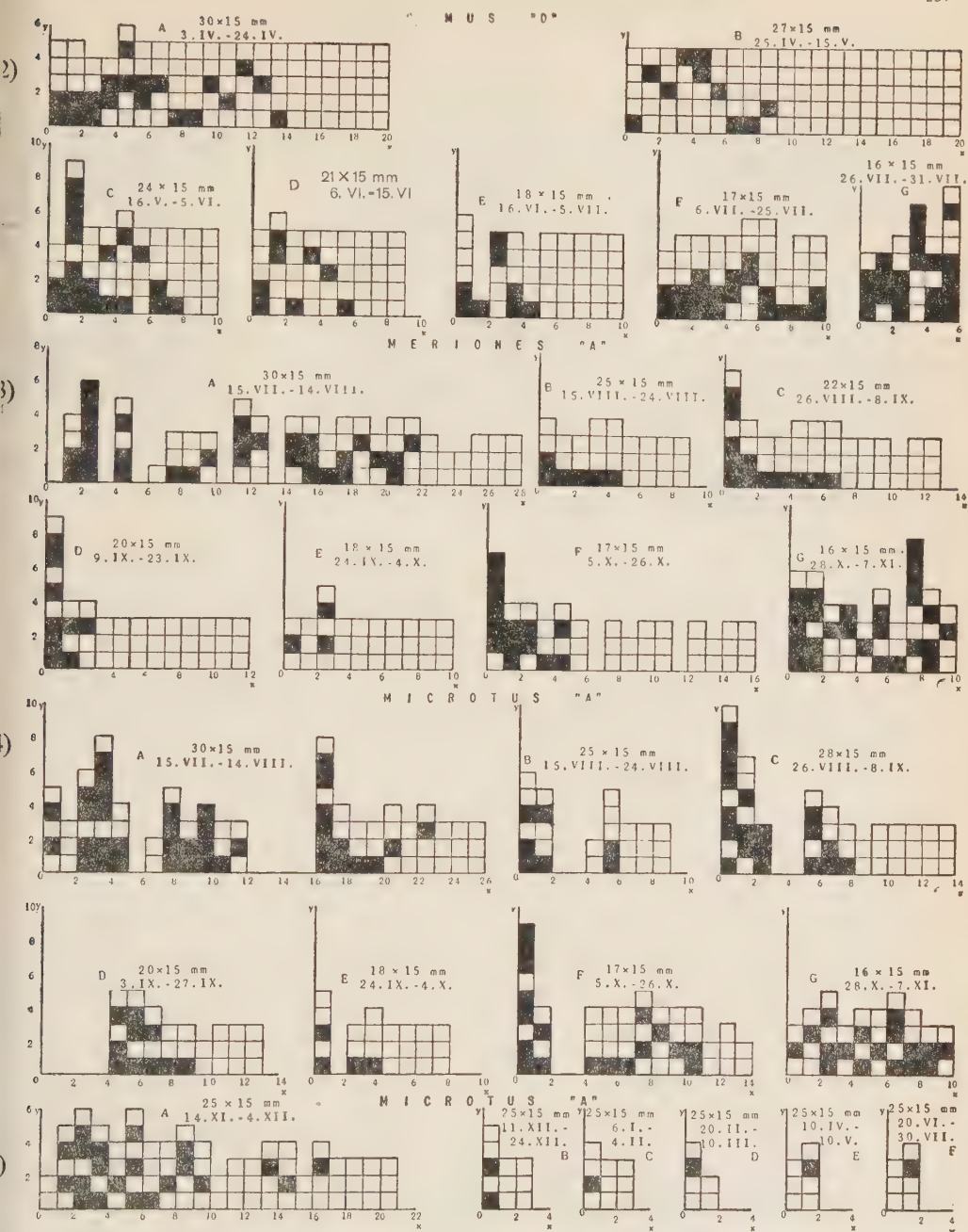


Figure 2. *Mus musculus* trained on square 15 × 15 mm and on rectangles of progressively decreasing base. x — days, y — number of experiments.

Figure 3. As Figure 2, for *Meriones tristrami*.

Figure 4. As Figure 2, for *Microtus guentheri*.

Figure 5. *Microtus guentheri* trained as in Figure 4, after the shock from its transport from Haifa to Jerusalem and a three weeks' interval since training.



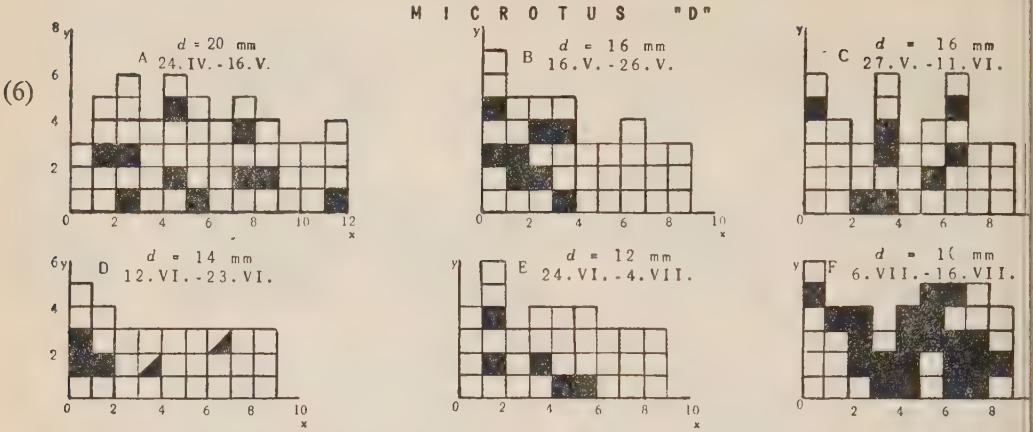


Figure 6. *Microtus guentheri* trained on circle of 10 mm diam. and larger circles of progressively decreasing diameters.

# CLAYS AND SOME NON-CARBONATE MINERALS IN LIMESTONES AND ASSOCIATED SOILS OF ISRAEL

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## ABSTRACT

The clay mineral composition of three different limestones and associated soils from Israel was investigated by X-ray analysis.

It is concluded that clay minerals of limestone derived soils are largely inherited from the parent material, and that the weathering of calcareous materials produces essentially no alteration of the clay minerals until the carbonates are completely leached out. The nature of the parent material and the properties of its clay minerals exert a dominant influence on the characteristics of the limestone derived soil in arid and semi-arid climates.

Some typical limestones were analysed for their insoluble non-carbonate residue and compared with clays separated from the overlying soils, in order to investigate to what extent clay minerals of soils associated with limestones are derived unchanged from the subjacent rock or are a product of pedochemical weathering. Some initial results of this investigation are reported.

The following three types of limestone, which generally give rise to widely different soils (Reifenberg 1947, Yaalon 1954), were selected:

- (1) a hard crystalline limestone associated with terra rossa;
- (2) a soft porous limestone producing a rendzinoid soil;
- (3) a marly friable limestone giving rise to a grey, highly calcareous mountain marl soil.

In the areas selected these limestones extend over a considerable portion of the landscape and sampling sites were chosen so as to exclude a possible admixture of alluvial material to the soil. The present climate is of the Mediterranean type, and essentially identical in all three sites.

## METHODS

The dissolution of the carbonates from the limestones was effected by an ammonium acetate—acetic acid solution adjusted to pH 3. This treatment is believed to be sufficiently gentle not to attack any of the clay minerals. The insoluble residue was separated by decantation and washed with ethanol—water mixtures.

Soil clays were obtained from the bulk of the soil by dispersion with Calgon (sodium hexametaphosphate) and separation of the less than  $2\mu$  equivalent spherical diameter (e.s.d.) particles by sedimentation.

\* Present address: Fertilizers and Chemicals, Ltd., Haifa.

X-ray photographs were taken with  $\text{CoK}\alpha$  radiation in a 9.0 cm diameter evacuated Bradley type powder camera capable of recording spacings up to  $50\text{ \AA}$ . Oriented aggregates were prepared by the method outlined by Brown (1953), and patterns were taken of specimens air dried, glycerol treated, heated at  $300^\circ\text{C}$  overnight and at  $500^\circ\text{C}$  overnight.

The clay minerals were identified at group level from the spacing of (001) reflections and their behaviour when subjected to the above treatment (cf. Brindley 1951).

The kaolin group minerals were identified by reflections at  $7.1\text{ \AA}$  and  $3.56\text{ \AA}$  unaltered by glycerol treatment or heating to  $300^\circ\text{C}$ , but which disappeared on heating to  $500^\circ\text{C}$ . Illite was identified by a line at  $10\text{ \AA}$  and higher orders, which were unaffected by any treatment. Minerals of the montmorillonite group were identified by a  $17.7\text{ \AA}$  reflection on glycerol treated specimens. With air dried specimens these minerals generally gave a broad band  $10\text{--}15\text{ \AA}$ , the width of the band being due to varying states of dehydration in the evacuated camera during exposure. On heating to  $300^\circ\text{C}$  and  $500^\circ\text{C}$  minerals of the montmorillonite group gave an asymmetric  $10\text{ \AA}$  reflection, with higher orders also broadened. Palygorskite\* was identified by its strongest line at  $10.5\text{ \AA}$  and higher orders, which were unaffected by glycerol treatment. The identification of this mineral was further confirmed by electron micrographs (Figure 1).

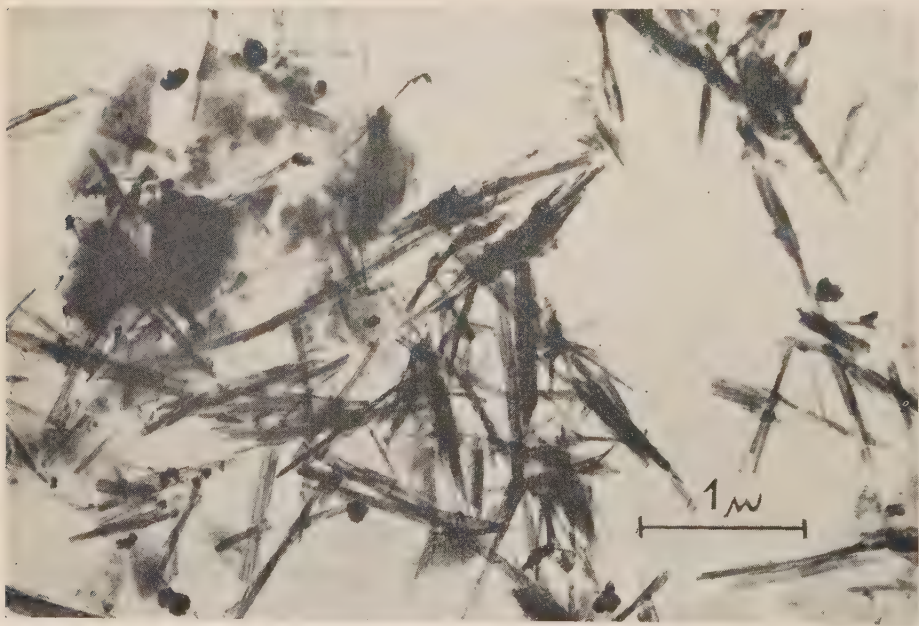


Figure 1

Electron micrograph of non-carbonate residue from Paleocene limestone, Safed ( $<2\mu$  fraction). The fibrous mineral is palygorskite, the fluffly mass on the upper left montmorillonite, and the small, dark irregular particles are probably quartz.

\* The name palygorskite has precedence over attapulgite; cf. Stephen (1954).



A rough semiquantitative estimate was obtained by visual comparison of line intensities and was supplemented in some cases by differential thermal analysis. The estimate of the 2:1 and 1:1 minerals was based on the intensities of the 10 Å and 7 Å reflections, assuming that the ratio  $I_{10}/I_7$  is 1/3 for equal amounts.

Non-clay minerals were also identified from the patterns of oriented aggregates. Quartz was identified from lines at 4.26 Å and 3.34 Å, but in the presence of illite only the former could be used due to coincidence. A strong line at 4.07 Å and a weaker one at 2.49 Å were attributed to  $\alpha$ -cristobalite. Goethite was identified by the 4.16 Å reflection which disappeared after heating to 300°C, and haematite by reflections at 3.67 Å, 2.69 Å, which often increased in intensity upon heat treatment. A reflection at 3.51 Å unaffected by all treatments was attributed to anatase. Reflections at 3.43 Å, 2.78 Å and 2.69 Å were attributed to carbonate—apatite, which has been subsequently identified optically in the coarser fractions of the residue as collophane.

A routine microscopical examination was undertaken of all the coarser fractions, and some of the minerals were isolated and identified by X-ray powder techniques.

## RESULTS

### *Terra rossa*

The hard crystalline limestone from the Jerusalem area is of Cenomanian age. In its purest state the limestone is greyish white and may contain less than 1% insoluble residue. On exposed weathered surfaces the stone acquires a reddish tint, and material accumulated in fissures is a bright red clay. The shallow soil overlying the rock is a typical terra rossa, generally almost lime free except for some rock fragments. The soil remains, however, base saturated and neutral in reaction.

The insoluble residue obtained by the ammonium acetate—acetic acid treatment was reddish brown in colour, not dissimilar from the terra rossa soil. Practically all of the residue was of clay size and most of it smaller than  $0.6\mu$  e.s.d. The dominant clay mineral was found to be illite (about 60%), with montmorillonite and kaolinite making up the rest. Judging from a line at about 13 Å obtained upon heating to 500°C, some interstratified chlorite layers are also present. Traces of haematite, goethite, quartz and anatase were also identified. Some of the bright red material was very easily dispersed with Calgon and proved to be amorphous to X-rays.

The silt and sand fractions of the residue contained mostly rounded quartz grains, some feldspar, collophane, limonite and a few resistant heavy minerals (zircon, staurolite, anatase and others) generally found in detrital residues.

The soil overlying this limestone contained an identical suite of clay minerals, although in different proportions. The proportions of illite and montmorillonite were reversed, and the latter was found to be dominant (70%), whereas the amount of kaolinite did not change appreciably. Traces of quartz, goethite, feldspar, and anatase were also identified. Another terra rossa soil from the Lower Galilee had essentially the same clay mineralogical composition.

The above composition is not necessarily typical for a terra rossa soil. Illite has been found to be dominant in some West African terra rossa soils (Munoz Taboada 1953), and it has also been suggested that kaolinite might be the characteristic mineral of these

soils (Muir 1951). The nature and extent of clay mineral weathering in red limestone soils thus must await future clarification. However, it appears that a large proportion of these minerals may be inherited or structurally derived from the parent rock, and that the amorphous constituents, although occurring in considerably smaller quantities than the crystalline material, determine to a considerable degree the essential properties of the resulting soil.

The red colour of these soils is usually attributed to free iron oxides, but generally none or only traces of haematite or goethite could be identified in the untreated soil clays. A characteristic feature, however, of all terra rossa clays so far investigated is the appearance of a strong haematite pattern obtained upon heating overnight at 700°C (G. Brown, personal communication). A weak haematite reflection is obtained at 500°C, but at higher temperatures the haematite lines often dominate the X-ray pattern.

Information regarding the quantities of some of the amorphous constituents may be obtained by various chemical means, but determination of their constitution is much more difficult. Their behaviour during weathering and their mode of association with the clay minerals also requires future clarification, and may be of greater significance than their absolute amounts.

### *Rendzina*

The porous limestone containing some cherty material, commonly occurring in the Bat Shlomo area, is of Lower Eocene age. These softer limestones give rise to a black rendzina-like soil, generally with a moderate lime content.

Almost all of the insoluble residue was of clay size and much of it was finer than 0.6 $\mu$  e.s.d. Its colour was greyish brown. The dominant clay minerals were montmorillonite and palygorskite, the former about twice as abundant. Some kaolinite was also present. Traces of quartz and  $\alpha$ -cristobalite were also identified in the clay fraction. The latter was also found to be present in chalcedony and siliceous earth isolated from the coarser residue, and in cherts and in fragments of siliceous earth in the overlying soil. Microscopic examination revealed the organic origin of this material. Feldspars and some heavy minerals typical of detrital residues were also identified.

The overlying soil contained montmorillonite as the dominant clay mineral, and small amounts of kaolinite (about 5–10%) were also present. No palygorskite was identified in the soil clay, and it appears that the leaching and loss of lime resulted in the decomposition of this mineral or in its alteration to montmorillonite.

When heated to 700°C the soil clays produced a distinct haematite pattern, similar to that observed on red clays. The organic carbon content of this soil is 1.7%, and is not significantly different from the organic carbon content of some terra rossa soils. When calcium-saturated, the organic matter is extremely stable and did not decompose when treated with hydrogen peroxide unless it was first acidified.

Montmorillonite has been generally reported to be the characteristic clay mineral of rendzinas and other black soils of arid or tropical climates with a distinct dry period (Mohr and Van Baren 1954). Singh (1954) suggested that the coloration in these soils is due to organic montmorillonite complexes. The formation of these complexes seems to be determined by the internal moisture regime of the calcareous rock and of the clay,

and is the result of the combination of a suitable climate, the porous nature of the rock and the swelling properties of montmorillonite.

### *Mountain marl*

The friable marly limestone from the Safed area is of Paleocene age (Shiftan 1952). It gives rise to shallow grey highly calcareous soils, named "Mountain marl" by Reifenberg (1947).

During dissolution the laminar structure of the limestone was clearly evident and it was observed that some of the clay was intimately associated with the lime, but that it also formed thin independent layers. Over 80% of the insoluble residue consisted of clay minerals and possessed a particle size distribution similar to the lime free residue of the overlying soil. The residue was considerably coarser than that from the other samples examined, and about half of the clay minerals were larger than  $1\mu$  equivalent spherical diameter.

The dominant clay mineral was found to be palygorskite, being about twice as frequent as the associated montmorillonite (Figure 1). Traces of quartz, cristobalite and carbonate—apatite were also identified in the clay and were dominant in the coarser fractions. The latter gave the same pattern as platy yellow grains of collophane isolated from the coarser residue. No heavy minerals were identified.

The clay isolated from the overlying soil contained mainly palygorskite and montmorillonite in approximately equal proportions. Traces of quartz and cristobalite were also identified. A significant difference was the presence of a small amount of kaolinite. The origin of this mineral in the soil is uncertain. The calcareous environment would not seem to be suitable for the formation of kaolinite, and the general appearance of the soil also indicates an essentially unaltered rock material. Aerial contamination is a possible source. No measurable kaolinite peak was obtained by differential thermal analysis, its amount is therefore likely to be less than 5%.

### GENERAL DISCUSSION

The presence of clay minerals in limestones has been established by numerous investigators. Illite and montmorillonite appear to be common in Paleozoic marine limestones, and kaolinite is more abundant in younger sediments (Grim et al. 1937, Robbins and Keller 1952, Schroeder 1952, Schachtschabel and Schroeder 1953). In many older investigations strong acid treatment was used to obtain the residues, which is likely to have destroyed montmorillonite and some of the other minerals. The results may therefore not represent the true distribution of clay minerals in limestones.

Palygorskite seems to be less common and is rare in strata older than Tertiary. It occurs mainly in association with lacustrine limestones, often as narrow bands of the pure mineral (Millot 1949). The particular environment conducive to the formation of palygorskite is not known, but it does not seem to be a product of atmospheric weathering. Its general association with calcareous sediments seems to indicate an authigenic formation as a chemical precipitate rather than a detrital origin. The absence of heavy minerals in one of the limestones is significant in this connection. Palygorskite of hydrothermal origin has also been reported (Caillere 1951, Stephen 1954).

Whenever present in soils palygorskite is likely to be inherited from the parent material and seems to indicate a moderate intensity of soil formation. The present investi-



gation provides evidence that it is less stable than montmorillonite. The few occurrences of palygorskite (attapulgitite) in soils reported in the literature are restricted to various calcareous soils of southern France (Michaud et al. 1945), desert soils of Syria and Persia (Muir 1951 and unpublished), and a rendzina soil in Australia (Rogers et al. 1954). In this last soil its origin has been traced to a palygorskite-bearing dolomite.

The evidence of inherited clay minerals in soils is of considerable significance in the understanding of soil genesis, especially in studies concerning the kind of climate or soil forming processes favouring a specific clay mineral. Clay minerals derived unchanged from various sedimentary materials are commonly found in soils of a wide variety of environmental conditions (Van Houten 1953). The proportion of such inherited clay minerals is likely to be larger in arid and semi-arid regions, where chemical weathering is greatly retarded.

Clay minerals are commonly the main component of the insoluble residue of limestones. The present investigation seems to support the conclusion that in many limestone derived soils the clay minerals are largely inherited from the parent rock, and that they are likely to have influenced the mechanism of the pedogenic processes. However, it is not intended to suggest that clay minerals produced by weathering are insignificant or that other soil forming factors are not important.

It appears that in the weathering of calcareous sediments there is essentially no alteration of the clay minerals, at least until the carbonates are completely leached out. Hence in base saturated soils, common in an arid or semi-arid environment, the clay minerals will be largely inherited from the parent material, and the nature of the rock and the properties of its clay minerals will exert the dominant influence on the characteristics of the derived soil.

As decalcification progresses other soil forming factors gain in importance and the clay mineral content may differ from that of the parent material. Thus, red and yellow podzolic soils derived from limestone seem to favour the formation of kaolinite, irrespective of whether the mineral was present in the limestone or not (Van Houten 1953).

#### ACKNOWLEDGEMENT

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# NOTES ON THE CLAY MINERALOGY OF THE MAJOR SOIL TYPES OF ISRAEL

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## ABSTRACT

The clay mineral composition of the major soil types of Israel was investigated by X-ray diffraction, and the results are tabulated.

Mineralogically the soils were divided into three groups: those containing montmorillonite and illite in various proportions with kaolinite as accessory, those containing kaolinite as the dominant clay mineral, and those containing palygorskite and montmorillonite.

The mineralogical composition is discussed in relation to the most likely origin of the clays. Only the clays of the basaltic and red sandy loam (hamra) are believed to be largely authigenic, whereas in all other soil types the clay minerals are inherited or structurally derived from the parent material, and are likely to have influenced the development and nature of the resulting soils.

The present account gives a brief summary of the mineralogy of the clay size particles in some soils of Israel selected to represent the major genetic soil groups of the region.

Several properties of the calcareous soils investigated in the present study were discussed in another paper (Yaalon 1954), and the samples used are from the same sites as those reported there. In addition some non-calcareous soils were investigated. The main characteristics of the soils are summarized in Table I. It is not suggested that each of the soil types listed is necessarily of great soil group status; some are great soil groups, but others are probably sub-groups.

A schematic distribution map of soils of Israel may be found in the works of Reifenberg (1947), Zohary (1947) and Ravikovitch (1953). At present the only data on the clay mineralogy of soils from the Eastern Mediterranean are those reported by Muir (1951) for Syria. In another paper the present author discusses the clay mineralogy of limestones and associated soils of Israel (Yaalon 1956).

## METHODS AND RESULTS

The soil clays were obtained from the bulk of the soil by dispersion with Calgon (sodium hexametaphosphate) and separation of the less than  $2\mu$  equivalent spherical diameter particles by sedimentation. Where the clay was found to be highly calcareous, the dissolution of the lime was effected by ammonium acetate at pH 3, a treatment which, in contrast to the generally used dilute HCl treatment, is believed not to attack any of the clay minerals.

\* Present address: Fertilizers and Chemicals, Ltd., Haifa.



Soil type	Locality	Parent material	Climatic classification*		Rain factor (Reifenberg)	Clay ( $<2\mu$ e.s.d.)** %	CaCO <sub>3</sub> ** %	Cation exchange capacity*** me/100g soil	Remarks**
			Type (Meigs)	Type (Meigs)					
Terra rossa	Jerusalem area	Hard limestone (Cenomanian)	Subhumid Mediterranean	60	59.2	6.25	42.0		
Terra rossa	Lower Galilee	Hard limestone (Cenomanian)	Subhumid Medit.	60	60.9	2.2	46.5		
Mountain marl	Safed	Marly limestone (Paleocene)	Subhumid Medit.	65	26.4	55.5	16.4		
Med. rendzina	Bat Shlomo	Soft limestone (Lower Eocene)	Subhumid Medit.	50	57.4	7.1	51.2	1.7% organic carbon	
Black Kabbara	Ma'agan Michael	Swampy calcareous alluvium (Upper Pleistocene)	Subhumid Medit.	40	38.0	24.1	40.2	4.6% organic carbon	
Lisan marl	Zemah	Lacustrine marl (Middle Pleistocene)	Semi-arid Sc 24	25	37.0	37.5	21.8	Exchangeable Mg increases with depth	
Loess	Beersheba area	Aeolian calcareous loess (Middle Pleistocene)	Arid Ac 23	15	14.2	23.8	9.8	Exchangeable Mg increases with depth	
Grey calcareous	Mishmar Hanegev	Alluvial + aeolian calcareous material (Middle Pleistocene)	Semi-arid Sc 23	25	15.1	12.9	12.0		
Red sandy loam (Hamra)	Rehovot	Dune sand, wind-sorted (Middle Pleistocene)	Semi-arid Sc 23	30	18.0	—	6.1	75% base saturated	
Basaltic clay	Lower Galilee	Basalt	Subhumid Medit.	60	53.3	—	38.6	88% base saturated	
Alluvial clay	Natufa valley	Terra rossa alluvium	Subhumid Medit.	55	65.5	—	51.2	94% base saturated	

\* The climatic classification of Meigs (Desert Research 1953) is based on Thornthwaite's moisture index for the definition of the major divisions; subdivisions are based on temperature: (24) indicates a hot summer, mild winter type; (23) stands for warm summer, mild winter type. Reifenberg's rain factor (1947) is, similarly to Lang's, the ratio between precipitation and temperature, but is based on the rainy winter season only.

\*\* Analytical methods: clay content of the carbonate-free sample by the pipette method, with Calgon as dispersant; CaCO<sub>3</sub> content by the volumetric Passon method; cation exchange capacity by ammonium saturation at pH 7; organic carbon by dry combustion.

X-ray photographs were taken with  $\text{CoK}\alpha$  radiation in a 9.0 cm diameter evacuated Bradley type powder camera capable of recording spacings up to 50 Å. Oriented aggregates were prepared by the method outlined by Brown (1953), and patterns were taken of specimens air dried, glycerol treated, heated at 300°C overnight and heated at 500°C overnight.

The clay minerals were identified at group level from the spacing of (001) reflections and their behaviour when subjected to the above treatments (cf. Brindley 1951). A rough semiquantitative estimation was obtained by visual comparison of line intensities and was supplemented in some cases by differential thermal analysis for the quantitative estimation of kaolinite. The semiquantitative estimation of the 2:1 and 1:1 minerals was based on the intensities of the 10 Å and 7 Å reflections, assuming that the ratio  $I_{10}/I_7$  is  $1/3$  for equal amounts.

Traces of non-clay minerals were also identified from the patterns of oriented aggregates. Quartz, cristobalite, haematite, goethite, calcite, feldspar and anatase are among those identified. The results are presented in Table II.

TABLE II  
*Clay mineralogical composition*

Soil type	Dominant (>50%)	Abundant (20—50%)	Accessory (5—20%)	Traces* (<5%)
Terra rossa (Jerusalem)	Montmorillonite		Kaolinite Illite	Goethite, Anatase, Calcite, Feldspar
Terra rossa (L. Galilee)	Montmorillonite		Kaolinite, Illite	Calcite, Feldspar
Mountain marl	Palygorskite	Montmorillonite	Calcite, Kaolinite	Cristobalite
Med. rendzina	Montmorillonite		Kaolinite	Cristobalite, Calcite, Feldspar
Black Kabbara		Montmorillonite	Kaolinite, Calcite, Palygorskite	Feldspar
Lisan marl		Montmorillonite Kaolinite	Illite, Calcite, Palygorskite	
Loess		Illite Montmorillonite	Kaolinite, Chlorite, Calcite	Anatase
Grey calcareous	Montmorillonite		Illite, Calcite, Kaolinite	Chlorite
Red sandy loam (Hamra)	Kaolinite	Illite	Montmorillonite	Haematite, Goethite
Basaltic clay	Montmorillonite	Kaolinite	Interstratified (12.5 Å)	Goethite, Anatase
Alluvial clay	Montmorillonite	Kaolinite	Illite	Chlorite, Anatase

\* Traces of quartz were present in practically all samples and are not listed.

## DISCUSSION

*Grouping*

From considerations of their mineralogical composition the soil clays can be divided into three groups.

The first group includes those soils which contain montmorillonite and illite as main constituents, with kaolinite as the accessory mineral. To this group belong the terra rossa, basaltic, loessial and alluvial soils. It includes soils with either a calcareous or basic igneous parent material. The clay content and the cation exchange capacity of these soils may vary considerably, but the exchange capacity of the clay seems generally to fall into the range of 70—80 m.e. per 100 g clay, supporting the X-ray observations that montmorillonite is generally the more important constituent of the clay.

To the second group belong soils which possess kaolinite as the dominant clay mineral. The red sandy loams (hamra) of the Coastal Plain appear to be the only ones belonging to this group.

The third group of soil clays that can be distinguished are those that contain palygorskite. Palygorskite is not known to be formed by soil forming processes; its presence would thus seem to indicate that the clay has been inherited from the parent material, and that the soil has not been subjected to strong weathering processes. Montmorillonite, which seems always to accompany it, is present in considerable amounts and may even dominate whenever the less stable palygorskite has been decomposed or altered by weathering. Illite and kaolinite are generally only minor constituents and may be entirely absent. The soils belonging to this group are the mountain marl, Lisan marl and, strictly speaking, also the rendzina soils, since their parent material has been shown to contain palygorskite (Yaalon 1956).

*Origin*

Differences or changes in the nature of the clay minerals are often thought to correspond with stages in the evolution of the soil. However, clay minerals present in surface soils are not always produced by pedogenetic weathering processes. Especially under conditions of a semiarid or arid environment, with its retarded chemical activity in the soil, the clay minerals are largely inherited or structurally derived from the parent material. Yet both authigenic and inherited minerals are subject to the various physical processes of movement and translocation, which are significant factors in soil development under these environmental conditions (Rim 1954).

Of the soils reviewed in the present study only the clay minerals of the basaltic and the red sandy soils are likely to be largely or wholly the product of atmospheric weathering. In agreement with the general observations of a suitable environment for clay mineral development (Grim 1953), the basic igneous basalt rocks favour the formation of montmorillonite, whereas the base poor coastal sand dunes give rise predominantly to kaolinite.

The composition of the limestone derived soils (terra rossa, rendzina and mountain marl) has been discussed in another paper (Yaalon 1956), and their minerals were shown to be largely derived or inherited from the clay minerals present in the parent rock. No single clay mineral seems to be typical for terra rossa soils; their essential characteristics seem to be more closely associated with the small amounts of free iron oxides.



It appears that in the weathering of these calcareous sediments no or little alteration of the indigenous clay minerals takes place, at least until the carbonates are completely leached out and the calcium removed from the environment. Some decomposition of the less stable minerals takes place and changes somewhat the relative proportions of the indigenous minerals, e.g. in the rendzina soil, which has lost practically all of the palygorskite present in the Lower Eocene limestone. The inherited clay minerals are likely to have influenced the mechanism of the pedogenic processes in these soils, especially in the rendzina soil, whose characteristic black colour is attributed to organic montmorillonite complexes.

Largely inherited clay minerals dominate also in the various alluvial soils. Their clay minerals give a good indication of the soils or areas which have contributed to the formation of the alluvium. This is evident, e.g., from the clay mineralogical composition of the black Kabbara soil, and even more so in the case of the grey calcareous or steppe soils of the Northern Negev. These soils were built up during the early Pleistocene from the alluvial downwash from the Eocene upland and intermixed with aeolian loess. It appears that the alluvial material supplied mainly montmorillonite, whereas the loess contributed illite. Various proportions of these two minerals are thus to be found in these soils, depending on the degree of admixture of loessial material (Soil Mechanics Laboratory 1952).

The Lisan marl soils of the Upper Jordan Valley and the loess soils of the Negev are both formed on deep Pleistocene deposits. The lack of clear morphological stratification and the textural uniformity with depth characterizing these soils would seem to indicate that only moderate soil forming processes were responsible for the soil development, and that no extensive clay mineral transformation of the native clay minerals is likely to have taken place. Muir (1951) attributed the lack of horizon development to the long-continued arid conditions and to the continuous renewal of the top soil by erosion. However, there is definite evidence of some chemical activity in these soils. The amount of exchangeable magnesium shows a consistent increase with depth (Yaalon, unpublished; Ravikovitch 1953). Calcium is generally by far the dominant exchangeable cation in calcareous soils, whereas exchangeable magnesium in such soils amounts to about 10–20% of the total exchange capacity. In the Lisan marl and the loessial soils its proportion increases with depth and at a depth of 1–1.5 m reaches about 40–50% of the exchangeable cations, which is often more than the amount of exchangeable calcium.

During the process of soil leaching, under natural rainfall conditions, replaceable magnesium becomes mobile with greater ease than calcium and may therefore develop such a gradient. However, the amount of leaching under the arid environmental conditions of these soils is restricted. Also the fact that these soils do not tend to develop a solonetz or solod morphology suggests that the leaching of salts is not responsible for this phenomenon. The author is therefore led to suggest that the Mg gradient is more likely connected with clay mineral processes which may eventually lead to the development of morphological horizons. Only a detailed profile investigation can indicate whether there are systematic differences in the clay mineralogy with depth which could be attributed either to weathering or to clay genesis processes, and thus prove or disprove the above hypothesis.

### *Non-clay minerals*

The red colour of soils is usually attributed to free iron oxides of various degrees of hydration. Generally only traces of crystalline iron oxide could be identified on the air dry patterns of the clays. Positive indications of haematite,  $\alpha\text{-Fe}_2\text{O}_3$ , and goethite,  $\alpha\text{-FeOOH}$ , were obtained in the red sandy loam, and of goethite in the terra rossa and basaltic soil. Overnight heating of the terra rossa soils at  $700^\circ\text{C}$  produced, however, strong haematite lines, indicating that at normal temperatures the largest part of the iron oxides is amorphous to X-ray. A similar reaction was obtained also on the rendzina clays, and seems to suggest that not the absolute amount of the iron oxides, but their behaviour during weathering and their mode of association with the clay determines the essential characteristics of the resulting soils.

Traces of quartz have been identified in all samples. Cristobalite, a high temperature modification of quartz, was identified in the mountain marl soil and its origin was traced to the parent rock (Yaalon 1956).

Anatase,  $\text{TiO}_2$ , is a common constituent of soils and was identified in several clays.

### ACKNOWLEDGEMENT

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# OCCURRENCE OF FOSSIL PHORONIDEA-LIKE TUBES IN SEVERAL GEOLOGICAL FORMATIONS IN ISRAEL

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## ABSTRACT

Straight, vertical tubes, 0.1—1.0 mm in diameter and up to 30 mm long, typically present in the Maestrichtian chalky, limonitic limestone of Israel, are supposed to be formed by *Phoronidea* of the genus *Phoronopsis*. *Phoronidea* colonies are characteristic for shallow, neritic marine environment, which corresponds well with the lithological properties of the Maestrichtian sediments, and they may indicate sedimentary or at least facial discontinuities. Such *Phoronopsis* facies appears in a less typical development and much less commonly in the Palaeocene and Middle Oligocene chalky limestones of Israel, always in a situation of sedimentary or facial discontinuity. This *Phoronopsis* facies is compared with that of "Craie du Meudon" of the Paris Basin.

In some conglomeratic horizons of Upper Cenomanian, Turonian and Pliocene, the pebbles are sometimes coated with tiny, tangled, irregularly spiral tubes which possibly may represent *Phoronidea* of the genus *Phoronis*.

In yellowish marly, limonitic chalk or chalky limestone of Maestrichtian age in Israel there can almost always be found small straight rods of limonite, 0.1 to 1.0 mm in diameter and from a few mm up to 30 mm long. In the bigger of these rods traces of tubular coating are often visible. The limonite of the rods is built concentrically. It is obvious that the rods constitute fillings of tubular organisms. These tubes are straight or slightly bent, standing vertically in relation to the bed surface. They are sometimes widely dispersed, but are mostly gregarious, numbering up to 10 rods per cm<sup>2</sup>.

The Maestrichtian marly layers containing the limonitic rods could be subdivided into two horizons: the lower one of coarser, granular texture, slightly phosphatic, stained with a few limonitic spots, with few or no limonitic rods, belongs, according to its foraminiferal fauna, to the upper portions of Lower Maestrichtian; it passes into an upper horizon of softer, fine-grained chalk, with many large, ramified or long, oval brown limonitic spots (which are possibly vestiges of *Porifera* activity) and with crowded, relatively long limonitic rods; this upper horizon has been proved to belong to Upper Maestrichtian.

Such limestones with the characteristic limonite rods were first observed in the vicinities of Hartuv and Kfar Uriah in the Shephela (Avnimelech 1936). Later they were encountered in almost the whole of Israel, wherever the corresponding horizon occurs; at Rosh Ramon at the south-west extremity of Makhtesh Ramon, at Hor Habar in the Zin valley, at Tel Yeroham, east of Beersheba, at Mishmar Ayalon, in a valley south-east of Shfeya, north of Sha'ar Ha'amaqim, at the foot of the Carmel, in Wadi Milh not far from Yoqne'am, in the Beit Netofa plain, etc.

In looking for the organisms which could be responsible for such vertical tubes, lower and higher plants, Foraminifera (such as *Bathysiphon*), *Porifera*, annelids, boring

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Mollusca, Pteropoda were considered, but none of these satisfied the requirements. Finally the living habitus and the tubes made by various groups of Phoronidea drew attention.

The Phoronidea represent a small group of marine animals whose proper zoological position is still uncertain. They include small worm-like, tubicolous, sedentary creatures, unsegmented, possessing a coelom (coelomata) and a characteristic lophophore. Their larvae are free-living, pelagic cosmopolitic, and are called *Actinotrocha*. In the mature stage they inhabit shallow sea bottoms, burrowing in mud, in limestone, in calcareous shells of thicker molluscs and in corals. They live in numerous associations of their own, but often also closely with small Porifera (*Cliona*), Actinia, some Bryozoa, Ophiura, Polychaeta and other sedentary organisms. Each individual is enclosed in a membraneous or leathery tube. The cylindrical elongated body varies in length from 1.5 to 127 mm. The Phoronidea as a whole have a wide geographical distribution. According to Cori (1937), they are euryhaline (sea water with 2.1 to 4.0% salts) and eurythermal. They inhabit the shelf region from about the surf-line to 50 m (30 fathoms) of depth. Their food consists of organic detritus, mostly Diatomea.

Two genera have been distinguished: *Phoronis*, which has a sinuous serpentiform tube and forms tangled colonies, typified by the colonies of *Phoronis kowalewskii* Caldwell; and *Phoronopsis*, whose tubes are straight or bent, forming vertical associations.

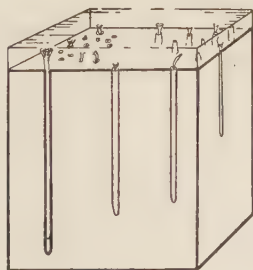


Figure 1

Diagrammatic representation of a colony of *Phoronopsis*. (After Fenton and Fenton 1934, slightly modified).



Figure 2

A portion of a tangled colony of *Phoronis kowalewskii*. Slightly magnified. (After Shipley 1910, modified).

The transparent, parchment-like tubes of Phoronidea in some species agglutinate sand grains, small shell fragments, etc. The tube is much bigger than the body of the animal itself. The accumulations often form a feltwork layer 5 to 8 cm thick (Shipley 1896) and, when boring in limestone, the animals convert its external part into a complicated system of canals. The boring is done with a solvent secretion of the animal.

Phoronidea are unknown as fossils because they lack a hard skeleton and because their tube is also incapable of fossilization. On the other hand, it is improbable that these so widely distributed organisms, whose present biological activity is so impressive and which phylogenetically are probably of quite remote origin, should have left no geological record. This problem was raised especially by Fenton and Fenton (1934), who attributed the common *Scolithus* tubes of the many early Palaeozoic sandstones to the activity of Phoronidea-like organisms. There are certainly many other formations

of different ages, where — together with other sedentary boring organisms — vestiges of the activity of *Phoronidea* are also to be found. A good example may be the famous and often discussed Craie de Meudon of the Paris Basin, near in age to the formation discussed here. Such a *Phoronidea* facies, obviously limited to a very shallow neritic zone, may be characteristic for the discontinuity surfaces in the case either of regression or of transgression.

The tubes in the Maestrichtian layers of Israel preserved as limonitic rods seem to be the vestiges of *Phoronidea* of the genus *Phoronopsis*. These layers can thus be appropriately and illustratively called "*Phoronopsis* marls and limestones". Such a designation will be not only associative with respect to the characteristic organic remnants, but will also serve as a stratigraphical as well as a facial indication. In fact, the *Phoronopsis* tubes ("*Phoronopsis* facies") are not encountered except in the said marly limestones of Maestrichtian (mainly Upper Maestrichtian) age, and also very rarely in the white chalky limestones of Lower Eocene age (as, for instance, in the western part of Wadi 'Ara) and not less rarely in the Oligocene (Stampian) limestone (Lakhish area). Thus they could be regarded as useful, easily recognizable index fossils of the Maestrichtian in Israel.



Figure 3

*Phoronopsis*-like tubes in a chalky limestone of Maestrichtian age (excavations at the Pumping Station near Daniel, east of Ramle).

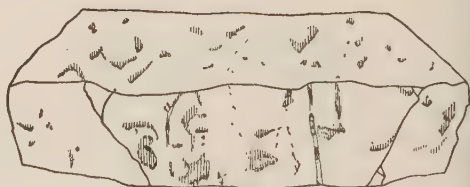


Figure 4

*Phoronopsis*-like tubes of Maestrichtian chalk (south of Shfeya).

The foraminiferal fauna of the Maestrichtian *Phoronopsis* limestone from several localities examined by Z. Reiss contains among others:

*Neoflabellina reticulata* (Reuss)

*N. coranica* (Marie)

*N. gr. efferata* Wedekind

*Allomorphina conica* Cushman and Todd

*Quadrinorphina allomorphinoides* (Reuss)

*Bolivina incrassata* Reuss

*Bolivina draco miliaris* Hiltermann and Koch

*B. draco draco* (Marsson)

*B. draco dorreeni* Finlay

*Loxostomum limonense* (Cushman)

*Eouvigerina* sp.

*Pseudouvigerina cristata* (Marsson)

*Buliminella laevis* (Beissel)

*B. cushmani* Sandidge

*Bulimina kickapooensis* Cushman and Parker

*Anomalina* (?) *pseudoacuta* Nakkady

*Pseudogümbelina excolata* (Cushman)

*Ps. costulata* (Cushman)

*Pseudotextularia* gr. *elegans* Rzehak

*Rugoglobigerina rugosa rugosa* (Plummer)

*R. gr. macrocephala* Bronnimann

*Globigerinella aspera* (Ehrenberg)

*G. messinae* Bronnimann

*Globotruncana lugeoni* Tilev

*G. stuarti* (Lapp)

*G. contusa* Cushman

*G. citae* Bolli

*G. mayaroensis* Bolli

*G. fornicata* Plummer

*Pseudovalvulinera gracilis* (Marsson)

*Stensiöina pommerana* Brotzen

Phoronidea tubes of less conspicuous occurrence are present also in some other formations in Israel, but there they are not of the straight *Phoronopsis* shape but in the form of tangled, irregularly spiral *Phoronis* tubes very similar to the living common species, *Ph. kowalewskii* Caldwell. These small complex tubes or canals can be found by the careful observer in several conglomeratic horizons of Upper Cenomanian age, or in similar formations (where present) in the base of the Turonian, or in the conglomeratic, calcareous sandstones of Lower Pliocene. In all these cases they coat with their complicated and inconspicuous mesh the included pebbles or other fragments. It is possible that their recognition could be decisive for differentiation between true and pseudo-conglomerates, which is in some cases quite difficult and uncertain. In any case, they are again characteristic as a witness of a discontinuity of sedimentation, whether on a very small or on a larger scale. Such tubes, of course, the shape of which is so primitive, may be attributed not only to Phoronidea but also to certain other organisms. However, their gregarious occurrence, and always in a facies which points to shallow neritic conditions, makes their attribution to Phoronidea at least very probable. Even admitting the doubts as to their true origin, it will be unquestionably useful to call attention to such tube-feltwork coating the pebbles in the different formations, and it will be convenient to call it "*Phoronis* coating". Such a descriptive name will help greatly in the characterization of pebbles included in rocks.

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# SOME PROBLEMS OF THE PRESENT DISTRIBUTION OF MOLLUSCAN SHELLS ON THE MEDITERRANEAN COAST OF ISRAEL

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## ABSTRACT

Mollusca found on the beaches of the Israel coast are surveyed and the dependence of their distribution on the bathymetric conditions of the littoral zone and on the morphological features of the coast is discussed. The geographical and geological origins of this fauna are considered and it is concluded that most of the species are of Miocene origin, mostly of subtropical character, and that from the Miocene up, the proportion of indigenous Mediterranean forms gradually increases.

## INTRODUCTION

To study the zoogeography and the geological past of the local Mollusca, shells were collected at 31 points along the 200 km long coast of Israel, beginning with Rosh Haniqra (Ras el Naqura) in the north and ending with Shiqma River (Wadi el Hessi), a few kilometres south of Ashqelon. In addition, material from shallow borings in the Haifa harbour was examined.

Besides the empty shells, representing the necrofauna, several amphibian genera such as *Littorina*, *Patella*, *Monodonta*, etc., were taken into consideration. A possibility exists that our necrofauna includes some ancient material derived by the action of waves from the sea bottom or from under the sand of the beach, such as the *Glycymeris* shells present in so called "*Pectunculus* beds", which may be of Pleistocene or even of Neogene age. This problem, however, has so far not been sufficiently clarified.

## PHYSICAL CHARACTER OF THE SHORES IN RELATION TO SHELL DISTRIBUTION

Theoretically we can assume numerous factors influencing the shell distribution along the shores, as for instance: 1) the distribution pattern of living mollusca in the nearest shelf region; 2) the mechanical properties of empty shells (size, shape, weight etc.); 3) the marine factors governing the dragging and transport of the shells toward the shore (gradient and morphology of the bottom, horizontal and vertical water movements etc.); 4) the character of the beach (its morphology and lithology, erosional features etc.). In practice it is hardly possible to define these factors on the basis of shell distribution except in a very general way.

For the purpose of our investigation four types of beaches are distinguished here:

a) *Quartz-sand beach*. Flat, low-gradient beach covered with fine, almost pure quartz sand or with coarser sand, containing rich shell detritus, mainly of *Glycymeris violacescens*. Such sandy beaches are widely distributed in the southern part from Gaza up to approximately Jaffa, while further north they are more and more limited

and discontinuous, being almost absent north of Carmel. On these beaches the shells accumulate in characteristic long, narrow strips, parallel to the coast line. Most of the shells are badly preserved and are broken and corroded from the protracted dragging across the sea bottom

b) *Lime-sand beach*. This beach covers most of the coast north of Acre. Here are found well ground mollusca detritus, foraminifera, echinoid spines, rounded calcareous algae, etc. (Avnimelech 1943). This lime-sand easily solidifies into gritstone, forming a hard, gradually dipping sea bottom and low longitudinal ridges on the shore. The shells found on these lime-sand beaches are much more variegated than those on quartz-sand, most probably because of the richer lime supply from the hills approaching the coast and because of the existence of many cavities and small bays.

c) *Sandstone cliffs*. Along the whole coast high calcareous sandstone cliffs, formed by abrasion of the old solidified dune-ridges, emerge in many points. This sandstone is the well known "kurkar". A narrow beach may be present, at the foot of the cliffs, but often the cliffs descend directly into the sea, leaving no such strip. The wave action is usually stronger in front of such cliff-sectors, and this often results in sporadic accumulation of shells, mainly of *Glycymeris violacescens*.

d) *Dolomite and limestone cliffs*. In the few sectors of the coast where mountains are close to the sea, i.e. at the Carmel Point and near the Lebanon frontier, the shores consist respectively of dolomite and limestone rocks descending steeply into the sea. There, characteristic surf manifests the strong action of waves. At Carmel Point the shelf between land and sea is comparatively broad, while it entirely disappears at Rosh Haniqra. In both places, the littoral strip is rich in cavities and crevasses, forming ideal nesting places for many marine organisms. In these parts characteristic amphibian mollusca together with colonies of *Serpula* and *Balanus* are always present. The most abundant of these mollusca are: *Mytilus minimus*, *Patella lusitanica*, *P. caerulea*, *Diodora graeca*, *Littorina neritoides*, *L. punctata*, *Monodonta turbinata*, *Purpura haemastoma*.

#### DISTRIBUTION OF THE SHELLS ALONG THE SHORE

The distribution of the 115 species of shells does not reflect directly the distribution of living fauna, since species with a hard and thick shell are preserved much better than those with a thin and fragile shell.

The designation of four categories — abundant, common, frequent and rare — used in the following distribution chart is of a rather general value. An exact statistical record would require a different approach to the problem, which was outside our scope.

The distribution of shells was found to differ with the season of year. The distribution chart demonstrates clearly the distinct differences between the southern and northern parts of the shore in correlation with their morphological and lithological character. The slight differences in the salinity and temperature of the sea water seem to influence the distribution of the fauna very little. The most important factor of this distribution seems to be the morphology of the continental shelf whose gradient is steeper in the north (beginning from Caesarea) than in the south.

## PELECYPODS

	W. Hasl	W. Ashqelon	Barnea	W. Sukreir	W. Rubin	Bat Yam	Jaffa	Tel Aviv	Herzliya	Arsuf	W. Falig	Natanya	Mikhmoret	Givat Olga	Caesarea	Tantura	Hahotrim	Tirat Hacarmel	Carmel Beach	N. of C.B.	Shiqmona	Bat-Galim	Haifa Harb.	Qishon	Bay of Acre	Naaman	Acre	Shavet Zion	Nahariya	Akhtiv	Geographical origin	Geological antiquity
1. <i>Nucula</i> (N.) <i>nucleus</i> (L.)																															Temp. M	1.
2. <i>Leda</i> ( <i>Lembulus</i> ) <i>pella</i> (L.)																															Tr. M	2.
3. <i>Leda</i> ( <i>Galactea</i> ) <i>lactea</i> L.																															Tr. M	3.
4. <i>Arca</i> ( <i>Navicula</i> ) <i>noae</i> L.																															Tr. M	4.
5. <i>Arca</i> ( <i>Barbata</i> ) <i>barbata</i> L.																															Tr. M	5.
6. <i>Glycymeris violascens</i> Lmk.																															Tr. M	6.
7. <i>Glycymeris pilosus</i> (L.)																															Med. M	7.
8. <i>Mytilus minimus</i> Poli																															Str. P	8.
9. <i>Mytilus thecaus</i> Gmelin																															Med. M	9.
10. <i>Musculus costatus</i> Risso																															Str. M	10.
11. <i>Mallevus regula</i> (Forsk.)																															Med. M	11.
12. <i>Perna</i> ( <i>Pinctada</i> ) <i>occa</i> (Reve)																															Med. M	12.
13. <i>Pecten</i> ( <i>Chlamys</i> ) <i>varia</i> (L.)																															Cosm. M	13.
14. <i>Spondylus gaederopus</i> L.																															Tr. M	14.
15. <i>Lima squamosa</i> Lmk.																															Tr. M	15.
16. <i>Anomia</i> (A.) <i>ephippium</i> L.																															Cosm. M	16.
17. <i>Astarte</i> (A.) <i>edulis</i> L.																															Str. M	17.
18. <i>Astarte</i> (A.) <i>sulcata</i> (da Costa)																															Temp. P	18.
19. <i>Cardita</i> (C.) <i>antiquata</i> (L.)																															Str. M	19.
20. <i>Cardita</i> <i>cabiculata</i> (L.)																															Tr. M	20.
21. <i>Begonia</i> ( <i>Glan</i> ) <i>trapezia</i> (L.)																															Tr. M	21.
22. <i>Loripes lacteus</i> (L.)																															Cosm. P	22.
23. <i>Cadokia</i> ( <i>Jagonia</i> ) <i>reticulata</i> (Poli)																															Str. M	23.
24. <i>Montacuta bidentata</i> (Montagu)																															Cosm. P	24.
25. <i>Chama gryphoides</i> L.																															Tr. M	25.
26. <i>Cardium</i> ( <i>Cerastoderma</i> ) <i>edule</i> L.																															Cosm. M	26.
27. <i>Cardium</i> ( <i>Rudicardium</i> ) <i>tuberculatum</i> L.																															Str. P	27.
28. <i>Dosinia lupinus</i> L.																															Cosm. M	28.
29. <i>Venus</i> (V.) <i>verrucosa</i> L.																															Cosm. P	29.
30. <i>Venus gallina</i> L.																															Cosm. M	30.
31. <i>Venerupis irus</i> (L.)																															Cosm. M	31.
32. <i>Petricola</i> (P.) <i>lithophaga</i> (Retzius)																															Str. M	32.
33. <i>Macrura corallina</i> L.																															Cosm. P	33.
34. <i>Mesodesma cornea</i> (Poli)																															?	34.
35. <i>Donax semistriatus</i> Poli																															Tr. P	35.
36. <i>Donax venustus</i> Poli																															Tr. Qu	36.
37. <i>Donax</i> ( <i>Capsela</i> ) <i>pollus</i> (Poli)																															Str. M	37.
38. <i>Tellina planata</i> L.																															Tr. M	38.
39. <i>Abra alba</i> (Wood)																															Cosm. M	39.
40. <i>Corbula</i> ( <i>Varicorbula</i> ) <i>gibba</i> (Oliv)																															Cosm. E	40.

Cosm. — cosmopolitic; Tr. — tropical to subtropical; Str. — subtropical to temperate; Temp. — temperate to boreal; Med. — exclusively Mediterranean; E. — Eocene; O — Oligocene; M — Miocene; P — Pliocene; Qu — Pleistocene.



## GASTROPODS

[illegible]

Cosm. — cosmopolitic; Tr. — tropical to subtropical; Str. — subtropical to temperate; Temp. — temperate to boreal; Med. — exclusively Mediterranean; E — Eocene; O — Oligocene; M — Miocene; P — Pliocene; Qu — Pleistocene.

GASTROPODS	W. Hasi	Ashelon	Barnea	W. Sukreir	W. Rubin	Bat Yam	Jaffa	Tel Aviv	Herzliya	Arsoff	W. Falig	Natanya	Mikhmoret	Givat Olga	Caesarea	Tantura	Hahotrim	Tirat Hacarmel	Carmel Beach	N. of C.B.	Shiqmona	Bat-Galim	Haifa Harb.	Qishon	Bay of Acre	Naaman	Acre	Shavei Zion	Nahariya	Akzhiv	Geographical origin	Geological antiquity
41. <i>Natica millepunctata</i> Lmk.																																M 41.
42. <i>Polynices</i> (Neritina) <i>joephepinus</i> (Risso)																																M 42.
43. <i>Trinia laryis</i> Blainville																																?
44. <i>Cypraea lurida</i> L.																																43.
45. <i>Cypraea spurca</i> L.																																Str.
46. <i>Cassidaria echinopora</i> (L.)																																Qu 44.
47. <i>Cassis saburon</i> (Bruguiere)																																Str.
48. <i>Dolium</i> (D.) <i>galea</i> (L.)																																M 46.
49. <i>Murex brandaris</i> L.																																Med.
50. <i>Murex</i> (Truncularia) <i>trunculus</i> L.																																Tr.
51. <i>Trophon</i> (Trophonopsis) <i>muri-</i> <i>catus</i> (Montagu)																																M 49.
52. <i>Tritonalia</i> (Ocinebrina) <i>edwardsi</i> (Payraudeau)																																Tr.
53. <i>Purpura faenastona</i> (L.)																																?
54. <i>Columbella scripta</i> (L.)																																M 53.
55. <i>Columbella rustica</i> (L.)																																Str.
56. <i>Cantharus</i> (Polia) <i>orbigny</i> (Payraudeau)																																M 54.
57. <i>Pisania maculosa</i> (Lmk.)																																Tr.
58. <i>Nassa</i> (Sphaeronassa) <i>mutabilis</i> (L.)																																M 56.
59. <i>Nassa incrassata</i> (Muller)																																Tr.
60. <i>Nassa</i> (Arcularia) <i>gibbosula</i> (L.)																																M 57.
61. <i>Nassa</i> (Arcularia) <i>circumcincta</i> Adams																																M 58.
62. <i>Nassa</i> (Hima) <i>reticulata</i> (L.)																																Tr.
63. <i>Fiasa</i> (Apyxis) <i>syracusanus</i> (L.)																																Cosm.
64. <i>Mitra ebanina</i> Lmk.																																Med.
65. <i>Mitra cornicula</i> L.																																?
66. <i>Cythara</i> (Raphiotoma) <i>brachystomum</i> (Phil.)																																Tr.
67. <i>Conus</i> (Lauticonus) <i>mediterraneus</i> (Brug.)																																M 62.
68. <i>Actaeon globulinus</i> Forbes																																Med.
69. <i>Ringicula conformis</i> Monterosato																																?
70. <i>Bullaria striata</i> (Bruguiere)																																Str.
71. <i>Cylichna</i> (C.) <i>cylindracea</i> (Pennant)																																M 69.
72. <i>Cylichna umbilicaris</i> (Montagu)																																Tr.
73. <i>Cylichna crossei</i> Boucauquoy, Dautzenberg, Dol.																																Cosm.
74. <i>Gadina garnoti</i> (Payraudeau)																																M 71.
75. <i>Siphonaria</i> (Williamia) <i>gussoni</i> (da Costa)																																Temp.

Cosm. — cosmopolitan; Tr. — tropical to subtropical; Str. — subtropical to temperate; Temp. — temperate to boreal; Med. — exclusively Mediterranean; E. — Eocene; O. — Oligocene;

In the following we would like to draw attention to the specific distribution of some of the more important species and to discuss briefly some of the resultant problems.

The comparative rarity of the genera *Nucula*, *Leda* and several others is connected with their generally deep or distant location in the sea. It is probably not a coincidence that the few specimens were found around Carmel Point, where the sea bottom descends more steeply.

The species *Arca barbata* is more common than *A. noae*, and it seems to be found most abundantly on rocky bottoms (beach south of Jaffa, Carmel shore).

The most abundant are shells of the genus *Glycymeris* often referred to as *Pectunculus*. It is represented on our shores by *G. pilosus* and *G. violacescens*. The first, which is present along the shores of Egypt and Lebanon, is restricted in Israel to the northern part of the shore, from Acre to the Lebanese frontier. *G. violacescens* is distributed in great quantities along the whole coast up to Haifa Bay, but from there on it becomes rarer. In Haifa Bay the commonly associated *Loripes lacteus* and *Cardium edule* become more frequent.

The bathymetric range of *G. violacescens* usually reaches up to 25 m. Judging from the great quantities of their shells on the beaches one might expect to encounter them in large quantities alive. Their source is not known with certainty. The Fisherman's Map of Rosenau (1938) indicates that at a depth of 30—40 fathoms and roughly parallel to the coast, there exist agglomerations of shells which according to Picard (1943) represent the so-called "*Pectunculus* beds". These shell accumulations are unidentified. They might be colonies of living *Glycymeris* or submarine layers of old "*Pectunculus* beds", which are present here and there in the coastal plain or encountered in the borings, and which belong mostly to some part of the Pleistocene or even to older horizons. *Glycymeris* shells accumulated on the beaches are mostly not well preserved, and their ligament is present in exceptional cases only, so that doubt arises as to whether some of them are not derived from older, fossil depositions. Such a suggestion may be also made regarding *Purpura haemastoma* and *Murex trunculus* which, although present in antiquity in high amounts and used for purple production, seem now to be much less abundant.

The very common *Mytilus minimus*, similarly to *Arca barbata*, is found mainly on rocky beaches (Jaffa, Arsuff, Caesarea, Carmel, Rosh Haniqra).

The uniform, although not abundant distribution of *Pteria* suggests that it flourishes along the whole shelf region of Israel, but not in the littoral zone itself.

*Spondylus*, and on a lesser scale *Lima*, are quite frequently found in the middle section of the shore, from Wadi Rubin to Caesarea, where the importation of the sand is smaller than in the south and the moderately covered rocky bottom creates for them favourable conditions. The occurrence of *Anomia ephippium* is similar but wider and probably depends on similar conditions.

The rare appearance of *Ostrea edulis* may perhaps be due to biological devastation and/or extermination by human activity in the past. There is some evidence suggesting its greater frequency in the past, at least in prehistoric times, especially north of Mt. Carmel. *Loripes lacteus* becomes more and more frequent along the Carmel shore and north of it.



*Cardium edule* and *C. tuberculatum* are very common along the whole length of the shore, but north of Mt. Carmel the more marine and stenohaline *C. tuberculatum* disappears, being replaced by the euryhaline and even brackish *C. edule*.

*Venus gallina*, like many other species, is more common in the vicinity of rocky beaches.

*Macra corallina* is frequently found along almost the entire shore line and is similar in distribution to *Arca barbata*. *Donax semistriatus* is common along the whole coast, but is more frequent along the Carmel shore. The limited occurrence of *Tellina planata* shells is possibly a result of their fragility.

As regards the distribution of Gastropod shells, the different conditions of their transportation by waves due to their mechanical properties should be taken into account. Owing to their shape they are more closely dragged and rolled upon the sea bottom, easily filled with sand or mud and thus are incorporated rapidly in the bottom sediments. This of course does not apply to such littoral, patelliform genera as *Diodora* and *Patella*, whose shells are left in immediate proximity to their habitat on the littoral rocks; accordingly they are encountered mainly in the central and northern parts of the shore. A similar pattern of distribution is that of *Monodonta turbinata*, due to its sessile habitat, mainly in the crevasses of the coastal rocks.

The small *Phasianella pullus* is frequent from the Carmel to the north, being rather rare in the southern and central parts of the shore.

The two species of *Littorina* are apparently evenly distributed in the rocky sectors of the coast.

An entirely different distribution is that of the genera and species of Rissoidae which are mostly accumulated along the Carmel shore and northward; it is probably influenced by a more favourable food supply (richer algal growth) and by the relative euryhalinity of this group. Similarly all the Cerithiaceae (*Bithium*, *Pirenella*, *Cerithium*, *Cerithiopsis*, *Triphora*) are more common in the northern than in the central sectors of the shore.

The delicate shell of *Janthina* are more common in the south than in the central and northern parts. The rare and very limited presence of *Aporrhais pespelicani* on the beaches illustrates its sporadic occurrence in the littoral zone of the sea. The even distribution of Naticidae along the whole coast is due to their relative independence of the character of the sea bottom. The Cypridae are — strangely enough — encountered most often in the middle sector of the coast. The Muricidae are distributed similarly to the Naticidae but *Murex trunculus* is more common than *M. brandaris*. The shells of *Purpura haemastoma* behave similarly.

*Columbella rustica* follows the ways of Cerithidae, being common mainly in the northern part of the coast. The distribution of *Conus mediterraneus* is similar, although this species is less abundant.

#### THE GEOGRAPHICAL DISTRIBUTION OF THE SPECIES

Almost all of the shells occurring on the coast of Israel are known to occur also on the Mediterranean coasts of Syria, Egypt, Tunisia, Algeria, France and on the Adriatic shores of Italy, and many of them on the Atlantic shores of Spain and Portugal.

According to the known distribution of the mollusca along the eastern Atlantic coasts and in the dependent seas (North Sea, Baltic, English Channel, Bay of Biscay, Mediterranean) we have divided the shells of the shores of Israel into the following climatic groups:

*Climatic groups of the shells of Israel shores*

Climatic groups	Number of species	% of the total
Cosmopolitic	21	18
Tropical to subtropical (occurring south of Spain)	35	30
Subtropical to temperate (zone from N. Africa to S. England)	33	29
Temperate to boreal (zone north of the above)	5	4
Exclusively Mediterranean	19	17

GEOLOGICAL HISTORY OF THE SPECIES

The mollusca under consideration are known as having made their earliest appearance in the geological times shown in Figure 1.

This account — even if we consider it with proper reservations — illustrates clearly the origin of the present mollusca of the shore, namely: no species were detected originating from pre-Cenozoic times: 1.7% only are of Paleogene origin; almost 98% of the present mollusca are of Neogene or younger origin. This is in accordance with the radical changes in paleogeography, climate, and general biological relations in the world as a whole and in the Mediterranean region in particular, which took place at the end of the Paleogene and at the beginning of the Neogene.

A more detailed analysis of these data will show how the molluscan fauna of our coast has developed during the ages. Let us consider at first the climatic compositions of the Miocene, Pliocene and Pleistocene elements of the present fauna as shown by the known paleontological data (Figure 2).

The mollusca of Pleistocene origin bear witness to the progressive stabilization of the Mediterranean elements, which increase their share up to 33% of the total, while the proportion of the tropical to temperate species remains almost unchanged (55%) but with surprisingly increased influence among them of more tropical species.

The cold-temperate species found in the Mediterranean today seem to be of an origin earlier than Pleistocene, and the boreal mollusca, which entered the east Mediterranean basin during the Pleistocene, have evidently not remained there.

The comparison of the three diagrams leads to the conclusion that the cosmopolitic elements have gradually withdrawn from the Mediterranean molluscan fauna, and their place had been systematically occupied by evolving indigenous Mediterranean fauna; the hot and moderately-hot elements remained relatively unchanged. This relationship illustrates the geographical-biological individuality of the Mediterranean basin gradually acquired starting from the Miocene till now.

Another approach to the same problem is represented by Figure 3, which shows the geological times of appearance of the cosmopolitic, subtropical-tropical, and Mediterranean elements of the present molluscan fauna, illustrating the gradual development of native Mediterranean elements.

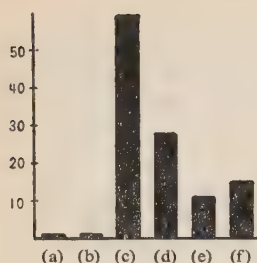
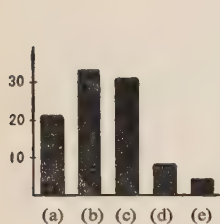
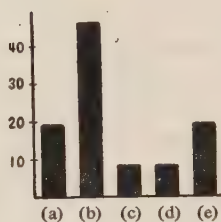


Figure 1

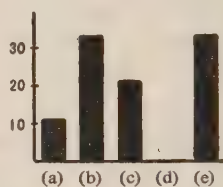
The antiquity of the present molluscan fauna on the Mediterranean coast of Israel. Appeared in (a) Eocene, (b) Oligocene, (c) Miocene, (d) Pliocene, (e) Pleistocene, (f) unknown.



(i)



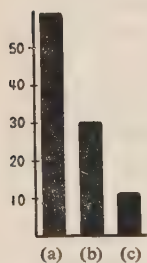
(ii)



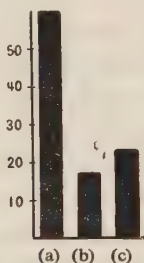
(iii)

Figure 2

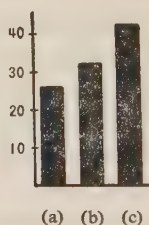
Zonal-climatic composition of (i) Miocene, (ii) Pliocene, (iii) Pleistocene elements in the present molluscan fauna. (a) Cosmopolitic, (b) Subtropical, (c) Tropical, (d) Cold, (e) Mediterranean.



(i)



(ii)



(iii)

Figure 3

Geological origin (times of appearance) of (i) Cosmopolitic, (ii) Tropical-subtropical, (iii) Mediterranean species. (a) Miocene, (b) Pliocene, (c) Pleistocene.

All the discussed diagrams show the great influence of the tropical-subtropical elements on the composition of the present molluscan fauna. These elements have been introduced to the Mediterranean mainly in Miocene time, but a great part has entered this region during Pleistocene. This fact is in good agreement with the late Cenozoic history of the Mediterranean Sea.

Although most of the mollusca we are dealing with are known as fossils from different countries of southern and western Europe, as well as from southern and eastern regions of the Mediterranean basin, only 20 species (17%) are so far known as fossils in Israel. This is probably caused by the insufficient knowledge of the marine Neogene and Pleistocene fauna of Israel, as confirmed by the fact that several species living today on the coast of Israel but not known there as fossils are reported in literature as fossils in the Neogene and Pleistocene of the Lebanon, Syria and Cyprus.



## CONCLUSIONS

The results of the present investigation might serve as illustration of the "future geology" of the coast. The following conclusions can be drawn from the distribution of molluscan and other remnants in the outcrops:

(1) The coast may be divided on the basis of molluscan distribution into the southern and the northern sections, the former being poorer in species than the latter.

(2) The northern section is richer in sessile mollusca as well as in species which usually dwell in deeper sea zones, thus bearing witness to a steep bottom gradient.

(3) Mollusca of several outcrops prove (together with the lithology of their stratum) the presence of rocky littoral zones rich in crevasses.

(4) The occurrence of *Glycymeris pilosus* north of Haifa and of *G. violacescens* south of this point could only be explained by different habits of these two species, the first living on a rockier and deeper bottom.

(5) The mollusca consist of 30% of tropical to subtropical species, a nearly equal proportion of subtropical to temperate species, only 4% of species of boreal origin, 18% of cosmopolitan species, and nearly the same relative amount of purely Mediterranean species.

(6) The mollusca do not contain elements older than Paleogene, the species of Paleogene origin consisting of less than 2% of the whole; 51% are of Miocene origin, 24% of Pliocene and nearly 10% of Pleistocene origin.

(7) The climatic origins of the mollusca of different geological times show that the Miocene had contributed most of the present tropical and subtropical species, while a second wave of hot species arrived during the Pleistocene. Colder-temperate elements penetrated into the Mediterranean in Neogene times, but those which entered the eastern Mediterranean in Pleistocene times have generally not succeeded in remaining there.

8) From the Miocene up an indigenous Mediterranean fauna steadily evolves.

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## LETTERS TO THE EDITOR

### The capacity of the hamster *Mesocricetus auratus* to produce agglutinins against *Leishmania* sp.

During an examination of the sera of hamsters with heavy infections of visceral Leishmaniasis produced by *L. tropica* and two strains of *L. infantum*, we were able to detect agglutinins against the flagellate stages of the homologous *Leishmania* only in one case; 1 out of 9 animals infected with *L. tropica* showed a titre of 1 in 10. The animals used in these experiments had been infected by intraperitoneal injection of L.D. bodies from the spleens of other hamsters in which the various strains were maintained by direct inoculation of L.D. bodies from infected spleen. It was therefore thought advisable to determine experimentally whether it is at all possible to obtain specific agglutinins against the flagellate stage of the Leishmanias in the sera of hamsters. Animals were subjected to six graduated injections of rich cultures of *Leishmania* sp. at intervals of 6 days and their serum was examined after a suitable period (10 days after completing a course of injections).

Three species of *Leishmania* were used for these experiments with the following results.

1) A strain of *L. infantum* (from Kenya) maintained in cultures on Locke-serum-agar for almost two years. The cultures were infective for hamsters and infected animals survived 6 to 9 months. Animals inoculated with the same strain maintained by direct passage from infected spleens survived 8 to 10 weeks.

Two series of 2 animals each were prepared, one with living cultures and the other with cultures killed in 0.5% phenol, centrifuged and washed three times in saline. In the first series the serum of one animal (no parasites found in the spleen and culture negative) had a titre of 1 in 50; the other (no parasites found in spleen smears but cultures positive) did not show agglutinins in dilutions of 1 in 5. The animals treated with phenol-killed cultures showed titres of 1 in 50.

2) A strain of *L. tropica* maintained for about two years on Locke-serum-agar. This strain produces visceral Leishmaniasis in hamsters relatively slowly. Hamsters prepared with this strain showed titres of 1 in 50 to 1 in 200.

3) A *Leishmania* sp. isolated by Dr. R. B. Heisch of Nairobi from the lizard *Latastia caudata*.

This *Leishmania* produces very high titres, 1 in 20,000 to 1 in 50,000 in rabbits. It is not infective for hamsters; out of 14 hamsters inoculated with rich culture material none became infected, although a culture was obtained from the spleen of a single hamster, on one occasion only, 6 days after intraperitoneal injection of flagellates (subsequent cultures from the same animal were negative; spleen smears were constantly negative). Hamsters prepared against this strain showed titres of 1 in 1000. (The hamster produces less agglutinins than the rabbit with all strains tested).

It is therefore clear that the hamster after repeated inoculation with material from cultures is capable of producing agglutinins against the flagellate stage of *Leishmanias*, and these agglutinins appear in the serum although agglutinins cannot be demonstrated in the serum of the majority of heavily infected animals in which reticulo-endothelial cells are stuffed with L.D. bodies.

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### Notes on breeding experiments with the albino strain of *Meriones tristrami* Thomas 1829

Tristram's jird, *Meriones tristrami* Thomas 1829, is distributed over almost the entire area of Israel. It inhabits mainly the lighter soils of the coastal plain, the Jordan and Bet Shean valleys, the slopes of hills and mountains in the north and centre of the country, and is abundant on the loess-loam soils of the northern Negev.

During the periodic mass outbreaks of field mice, an increase in the numbers of *Meriones tristrami* is generally observed, often resulting in considerable damage to field crops.

#### Breeding technique

The animals are housed in a well ventilated room on the ground floor of a concrete building. No artificial ventilation or heating appliances are used. The temperature ranges from about 13°C during the coldest months of the year to approximately 26°C in midsummer. The rodents are raised in sheet metal cages covered with a lid



made of wire netting. The dimensions of the cages are  $80 \times 40 \times 25$  cm. For bedding, wheat or barley straw or hay are used. The cages are cleaned thoroughly once a fortnight. The daily ration of adult animals consists of 8 g barley, 20 g carrots or beetroot and 40–50 g alfalfa, clover or other fresh herbs.

### History

In May 1950 two albino jirds were trapped in fields of the settlement Safiah, 28 km N.W. of Beersheba; in the course of the following 3 months hundreds of wild type coloured jirds were trapped in the fields of the settlement and the neighbouring countryside.

A large proportion of the trapped *Meriones* were killed in the course of laboratory tests with various chemicals, and only a limited stock was kept for observation and breeding experiments.

On 10.11.51 a litter of 2 female and one male albino jirds was born of a pair of *Meriones* of which the female (No. 27) had been trapped at Mishmar Hanegev (12 km south of Safiah) and the male (No. 80) at Safiah. After weaning, the litter was separated from the parents and given the serial numbers 171 ♂, 172 ♀, 173 ♀. The three animals were kept in the same cage, and the matings were therefore of the full brother-sister type.

The first litter of 172 ♀, born on 5.5.52, consisted of 8 young of wild type colour. A second litter of 4, of which 2 were albinos, followed on 2.7.52.

The first litter of 173 ♀, born on 7.8.52, consisted of 2 young of the wild type colour. The second litter, born on 7.9.52, consisted of 4 young — 2 albinos and 2 wild type variety.

The ratio between wild and albinos born of wild parents approaches very closely the Mendelian ratio of  $3/4 : 1/4$ , suggesting that the difference between the two colours is due to one pair of autosomal genes with complete dominance. The recessive gene, as is usually the case in mammals, is the one for albino.

### Fertility and growth

In spring 1953, when the albinos had reached sexual maturity, we commenced breeding experiments.

The initial pairings were not successful. In 4 cases the females were killed by the males and one male was killed by a female. Only in summer 1953 were the first pure litters of albinos born through inbreeding of brothers and sisters.

To date, August 1955, a large stock of pure albinos has been established, reaching  $F_5$  in our breeding of generations. Fifteen fertile females of various generations ( $F_1 - F_4$ ) have been under constant observation and the data collected are given below.

Of the above females, one gave birth to 13 litters in the course of 523 days, while from another female we obtained 11 litters in the course of 476 days. Two others gave birth to 9 litters (607 and 670 days respectively) and one female to 6 litters (285 days). The other females

TABLE I

♀ No.	Total No. of litters	Litters of wild type variety	Litters of albino and wild type	Total No. of young	No. of young of wild variety	No. of albinos	Died before colour determined
172	23	10 (43.4%)	13 (56.6%)	88	65	20	3
173	20	7 (35%)	13 (65%)	77	56	19	2

TABLE II

Month	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
No. of litters	6	6	5	9	10	10	10	10	5	4	5	4

TABLE III  
Average body weight (g)

Age (days)	1	25	50	100	200–250	500	No. of specimens
Albino ♀	3.1	25.4	39.6	79.2	87.0	101.0	30
Albino ♂	3.1	31.0	65.5	105.7	120.3	138.0	30
Wild type ♀	3.3	25.0	54	84.5	98.0	111.0	18
Wild type ♂	3.3	30.0	70	96.5	115.0	139.3	15

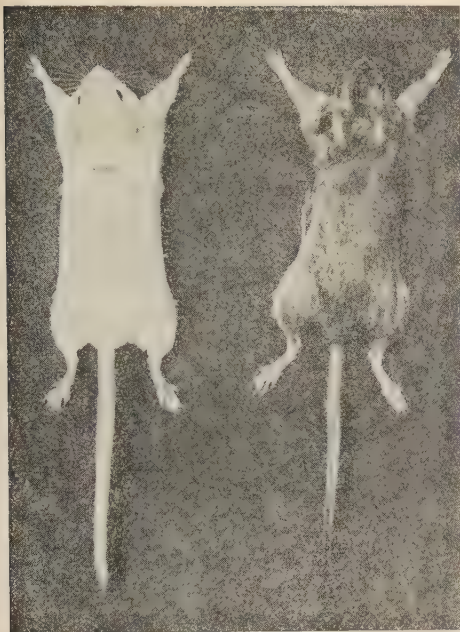


Figure 1  
*Meriones tristrami* Th. (x 1/2).  
Left: albino. Right: wild coloured animal.

have so far had from 2 to 4 litters each. Altogether 75 litters with a total number of 267 young were produced by the 15 females under observation. In none of the litters did individuals of wild type colour occur. Average number of young per litter was 3.5. Average interval between litters in permanently coupled pairs was 49 days. The sex ratio of the young produced by these females was 151 (56.6 %) females and 107 (40 %) males. Nine (3.4%) young died before the sex was determined.

Fertility was highest during the summer months (April — September) and somewhat reduced (but not interrupted) in winter. Of 86 litters, 51 were born during the 5 summer months (an average of 10.2 litters per month) and 35 during the other 7 months (average of 5 litters per month).

The young are born blind and naked. On the 4th day the first pelage of white hairs begins to show. The development of the young does not differ from that of the wild variety of *Meriones tristrami*<sup>1</sup>.

Though the table of weights is as yet incomplete, from the data at our disposal it can be seen that the average body weight of the albinos conforms to that of the wild variety.

### *Meriones tristrami* as a laboratory animal

In the course of our breeding experiments with the albino strain we found that these rodents exhibit qualities which may possibly prove their suitability as laboratory animals: a high rate of fertility, docility, convenient size and inexpensive maintenance; they do not bite and are most easy to handle. A large number of these species has been extensively used by I. van der Hoeden for tests on the pathogenicity of several serotypes of *Leptospira* to *Meriones shawi*<sup>2</sup>.

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### Two rare fishes from Eilat (Gulf of 'Aqaba)

This is a report on two fishes collected in Eilat in 1954. One of them, *Anyperodon leucogrammicus*, was not reported hitherto from the Red Sea, the nearest locality previously recorded for this species being Zanzibar.<sup>1</sup> The other species, *Barchatus cirrhosus*, was described by Klunzinger<sup>2,3</sup>, who had apparently but one specimen from the Red Sea (most likely from Koseir). No more specimens have been reported since. The finding of a specimen of this species in the Gulf of 'Aqaba adds to our knowledge of the distribution of this rare fish; its description may serve as a supplement to Klunzinger's original account.

### SERRANIDAE

*Anyperodon leucogrammicus* (Cuvier and Valenciennes)

*Serranus leucogrammicus* Cuvier et Valenciennes, 1827, Histoire naturelle des poissons, vol. 2, p. 347 (loc. : Malayan Archipelago).

One, A 302: 325 mm standard length. Eilat, 11.VI.1954, coll. A. Ben-Tuvia.

The absence of teeth on the palatine separates this genus from other genera of the family. Our specimen does not differ substantially from the description given by Schultz<sup>4</sup>. It should be mentioned that the length of the caudal peduncle exceeds its depth; the third anal spine is longer than the second, which is in contradiction to the data found in the key of Weber and de Beaufort<sup>5</sup>. The range of this species given by Fowler and

Bean<sup>1</sup> (vol. 3, p. 295) is "Zanzibar, Madagascar, Seychelles, East Indies, Philippines, Melanesia, Micronesia".

D XI, 15; A III, 9; P 15; V I, 5; lateral scale rows 96; scales from origin of anal to soft dorsal 27(1)16; gill rakers on the whole first arch 12(1)17.

Depth of body to standard length 3.1; head 2.3; postorbital 3.8; length of pectoral 4.9; length of ventral 5.2. Snout to head length 4.2; interorbital 7.9; eye 6.8; upper jaw 2.1. Third dorsal spine 3.3; third, fourth and fifth dorsal spines subequal; longest dorsal ray 2.6; depth of caudal peduncle in its length 1.3. Third anal spine 4.1; third anal spine the longest but not as strong as the second. Gill rakers longer than gill filaments, their lengths 1.2

Colour in formalin: brownish with dark brown spots all over the body, spots smaller on sides of head; top of head blackish; dark spots on dorsal and caudal fins, no spots on other fins. No pale streaks along body sides as commonly described for half-grown specimens.

Remark: the fish is preserved in the British Museum of Natural History, London (No. 1955.9.25.7).

#### BATRACHOIDIDAE

##### *Barchatus cirrhosus* (Klunzinger)

*Batrachus cirrhosus* Klunzinger, 1871, Synopsis der Fische des Rothen Meeres, p. 500 (loc. Red Sea).

One, A 232a: 220 mm standard length. Eilat, 27.II.1954, in trap. Coll. A. Ben-Tuvia.

Klunzinger gives the length of this fish as 34 cm. J. L. B. Smith<sup>6</sup> in his revision of the family assigns the species to a separate genus.

D III, 18; A 13; P 24; V I, 1; gill rakers 9.

Depth (in front of dorsal origin) 3.7 to standard length; head (measured to end of opercular spine) 2.4; predorsal 2.5; postorbital part of head 3.7; length of pectoral 3.7; of ventral 4.0. Snout to length of head 3.7; eye 6.0; interorbital 2.5; upper jaw 1.7; longest dorsal and anal rays subequal, 2.3; length of caudal peduncle 5.2, its depth 4.2. Maxilla reaching to behind eye border. Predorsal distance equal to length of soft dorsal base. Anal fin base subequal to postorbital part of head and somewhat longer than upper jaw. Skin-embedded small scales cover the body; head naked. Two strong spines on operculum, two on preoperculum. A conspicuous, fringed flap above the eye, another behind the corner of the mouth. Numerous small skin filaments on various parts of head. Gill rakers short knobs with 2—5 strong spines on each, on lower part of gill arch only.

Colour in formalin: marble-like pattern with irregular brown blotches on body. Brownish dots and streaks on fins and branchiostegal membrane.

Remark: the fish is preserved in the British Museum of Natural History, London (No. 1955.9.25.15).

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#### Occurrence of *Discoglossus nigriventer* in Israel

*Discoglossus nigriventer* Mendelssohn and Steinitz (Anura, Discoglossidae) was discovered in 1940<sup>1</sup>, when two frogs were found on the same day in March in a very circumscribed area on the eastern shore of Lake Hula. In August of the same year two tadpoles were found close by the same site. Although the areas in which those four specimens had been taken were searched fairly thoroughly for several years thereafter, no more specimens were found (the eastern shore of the lake has not been reinvestigated since 1947).

On May 20, 1955, Mr. M. Costa caught an adult frog of the same kind in the Hula swamp where a large scale land reclamation scheme is in progress (grid reference 205.280, Israel Survey Map). Mr. Costa has generously presented the specimen to this department. The animal, HU.AMPH. 544, measures 80 mm from snout to vent, being just twice as large as the specimen described in 1943<sup>1</sup>. The present frog is probably a female as concluded from external characters. Body proportions and colour pattern are much the same as described previously. Deviations due to the larger size and higher age of the animal are insignificant.



The frog was kept under close observation for about a week prior to its preservation. I am indebted to Mr. Costa for the following notes. The animal was kept in a tank whose bottom was covered with a layer of sand up to 5 cm deep; a little water was added, leaving only the highest parts of the sand layer uncovered. The frog showed a strong inclination to dig in the sand making use chiefly of its forelimbs. In daytime the animal was frequently dug-in, but with its head projecting above the surface of the shallow water. Activity was considerable only after night-fall.

This seems to confirm that, notwithstanding its scarcity, *Discoglossus nigriventer* is firmly established in this country, probably confined to a very limited area.

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### A scorpion *Leiurus quinquestriatus* H. et E. with two stings

A subadult specimen of *Leiurus quinquestriatus* H. et E. was found by one of us (P.A.) on 15.V.55 in Jerusalem.

At close examination it appeared to have two stings on its last metasomal segment (Figure 1), the ampula being somewhat invaginated from the left side and its terminal prolongation extended in a slight bend to the left. The right aculeus appears to be the direct prolongation of the ampula and the left one springs from its side some 2 mm from its apex.



Figure 1

Last metasomal segment of *Leiurus quinquestriatus* from the left side.

It seems that the cases of duplication (or clefting) of the metasomal segments were known to Plinius who describes a scorpion with two tails<sup>1</sup>. Pavesi<sup>2</sup> described *Euscorpium germanus* C.L. Koch in which the split into two reached the fourth segment of mesosoma. Sergent<sup>3</sup> described *Buthacus leptochelys* (H. et E.) with the body bifurcated from behind up to fifth segment of mesosoma. Vachon<sup>1</sup> brought the case of an adult *Androctonus crassicauda* OL. with two completely divided tails. Berland<sup>4</sup> described a young *Centruroides infamatus* Berland, whose tail was divided in two, starting from its second segment. The scorpion found by us showed only a slight development in this direction.

No adequate explanation of this phenomenon is yet known. Brauer<sup>5</sup> studied the structural abnormalities in the embryos of scorpions and suggested that the division of the metasoma and of the mesosoma may be caused by the division of the germ band or by secondary melting of the dissociated elements.

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### On two rare genera of ticks of domestic stock in Israel\*

The tick fauna of domestic stock in Israel is mainly composed of species belonging to the genera *Hyalomma*, *Rhipicephalus*, *Boophilus*, and *Haemaphysalis*, which thrive both in Europe and in Africa.

Ticks of the genera *Ixodes* and *Amblyomma* have rarely been found in this country, *Amblyomma* being an African genus and *Ixodes ricinus gibbosus* (the only species of this genus found on domestic stock in Israel) had been considered to be confined to Greece and Turkey.

With regard to the genus *Amblyomma* Hoogstraal (1954) writes: "*Amblyomma* ticks are absent in the Near East".

\* This investigation was supported by Research Grant No. E-1006 from the National Institutes of Health, U.S. Public Health Service.

Recently three males of *Amblyomma* were sent to us for identification by A. Hadani who had caught them in October, 1955, on three different cows near Rosh Pina (in the northern part of Israel). All three specimens were *A. lepidum* Dönitz, 1909.

*A. lepidum* had previously been caught only once by officials of the Department of Agriculture of the Mandatory Government on cattle, in Southern Palestine. In addition, several nymphs and one male which emerged from one of them, were taken off *Asio flammeus flammeus* (Strigidae) in Jerusalem on March 12, 1938, and are now in the collection of the Department of Parasitology. They were determined by Theodor as *A. lepidum*.

The above seems to be the northernmost record of this species and it is especially noteworthy since the species is not recorded from Egypt and its northernmost record had been the Anglo-Egyptian Sudan. The importation by cattle from Eastern Equatorial Africa is very improbable, since no cattle have been imported from there for many years.

The genus *Ixodes* is represented on domestic stock in Israel by *I. ricinus gibbosus*, *I. theodori*, the only other species known in Israel, having never been found on domestic stock. *I. ricinus gibbosus* was first described by Nuttall in 1916 from specimens taken off *Capra hircus* in Smyrna, and is also known from Greece (Pantazi). The distribution of this subspecies is probably much more extensive than indicated by records, since it is apparently often diagnosed as *I. ricinus*, the subspecies much resembling the type. Smith (1931) states, e.g., that the genus *Ixodes* is represented in Palestine by *I. ricinus*. Re-examination of his material\* shows that it is not *I. ricinus* but its variation *I. ricinus gibbosus*.

In Israel, *I. ricinus gibbosus* is mainly a parasite of sheep and goats, but we have one record from a donkey and one from a mule. The ticks are found in small numbers mainly in December—January, but sometimes also in November and February. All specimens were found in the hilly regions of the various parts of the country (Judea, Samaria and Galilee).

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#### Preliminary observations on the inheritance of some racial characteristics in drones of *F<sub>1</sub>* hybrids of *Apis mellifica* var. *ligustica* × var. *syriaca*

Unfertilized *ligustica* queens were introduced into a region in Israel inhabited by the local *syriaca* race. The segregation of drone phenotypes, observed in subsequent breeding, indicates that hybridization has occurred.

*Ligustica* drones exhibit a variable abdominal colour pattern, but the yellow pigmentation is, generally, predominant. Goetze<sup>1</sup> proposed for such drones a classification of six types. The knowledge of tergite colouration of *syriaca* drones is rather scanty, and no detailed analysis has so far been attempted. Some preliminary observations seem, however, to point to a great stability of the pigmentation pattern. In most cases only one tergite (the third, if counting the propodeum first) shows bright patches, free from melanin. Similar patches on the fourth tergite are only seldom encountered. The colour of the scutellum is yellow in *ligustica* drones and dark in Syrian ones, whereas it is invariably yellow in the workers of both strains.

The mother of the stock under observation was a certified pure Italian (*ligustica*) queen imported from the U.S. Mating with *syriaca* drones must have occurred. Only the male descendants of the *F<sub>1</sub>* generations were analyzed. These were obtained either by inducing workers to lay eggs<sup>2</sup>, or from an unfertilized queen. No measures were taken to prevent the infiltration of foreign drones, since such were only rarely seen in the stocks. The observations were carried out in 1952 and 1953 and are summarized in Table I.

TABLE I

Segregation of the colour of the tergites and of the scutellums in drones, descendants of hybrid queens or hybrid workers

Observation year	C o l o r a t i o n				Total no. of drones	Method
	Dark		Yellow			
	Terg. Scut.	Terg. Scut.	Terg. Scut.	Terg. Scut.		
1952	117	—	121	—	238	Lying workers
1953	52	52	49	48*	101	Unfertilized queen

\*A single yellow drone with black scutellum was found. In all other drones the colour of the scutellum and the tergites was the same.

These data show clearly that the drones, produced by hybrid females, segregate into two classes according to the colour of the scutellum and of the tergites in the ratio of 1 : 1. They also indicate that a positive correlation exists in this case between the pigmentation of the abdominal tergites and that of the scutellum.

The pure strains differ widely in their pre-cubital index (as defined by Goetze<sup>3</sup>). *Ligustica* drones have a mean index of 4.2 (range 4.1—4.25) while *syriaca* drones have one of 6.1 (range 5.4—7.6)<sup>4</sup>. Both classes of drones produced by hybrid females were found to possess a mean index of 4.3 which is practically identical with the index of var. *ligustica*.

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### A yellow bacterium isolated from banana but pathogenic to tomato and pea\*

Diseased or degenerated banana plants, examined in Israel from April 1954 to the middle of January 1955, were found to harbour many various bacterial organisms in the discoloured vessels of the partially darkened and often rotted trunk, base of the leaf, and bulb. Though none of these organisms, isolated in pure culture, produced any infection when inoculated on banana, it was interesting to note that at least two of these organisms were pathogenic to other hosts. The first organism, a green fluorescent bacterium identified as *Pseudomonas polycolor* Clara or *Ps. aeruginosa* (Schroeter) Migula and isolated during the summer and autumn, has already been described<sup>1</sup>. This paper describes a yellow bacterium, isolated during the months of October to the middle of January, from the base of the leaves of several banana plants.

Unlike the green fluorescent organism, the yellow bacterium is a wound parasite with a limited host range, and with markedly lower temperature relations. Inoculation of healthy unripe fruits or leaves was made, as described for the green fluorescent bacterium<sup>1</sup>, on the following hosts: avocado, lemon, grapefruit, orange, tomato and pepper fruits; pea-pods; tomato, potato and lettuce leaves; slices of various vegetables in water. The fruits and the leaves were kept in bell-jars over water at a temperature of 14°—37°C.

Of the inoculated hosts only tomato fruits and

pea-pods were infected. The lesions appeared as deeply sunken dry, necrotic, brown large spots on tomato fruits, and smaller, light-brown, slightly sunken, necrotic spots on pea-pods. The optimal temperature for infection was between 20° and 25°C. Infection was good at 14°C, slight at 30°C, and none at 34°—37°C. Experiments with re-isolations of all fruits gave similar results. The re-isolated organisms were identical with the original isolations. Histological sections showed numerous bacteria in the parenchyma tissue of infected parts. No infection resulted when drops of suspension were not pricked into the fruit or pod. Controls pricked through drops of sterile distilled water did not develop any sort of infection.

Examination of the organism shows that it belongs to the family Enterobacteriaceae Rahn<sup>2</sup>, genus *Erwinia* Winslow et al.<sup>2</sup>. It is a Gram negative short rod 0.3—0.45 x 1.5—2.4  $\mu$ , occurring singly and in pairs or short chains, motile with numerous peritrichous flagella, non-spore-forming, aerobe and facultative anaerobe. Optimum growth occurs at 25°C, maximum temperature is 39°—40°C, and minimum 3°—4°C, with thermal death point approx. 54°—55°C. Colonies on nutrient agar 48-hr old are circular, raised, glistening, with a net-like structure in the centre and granular rinds, deep yellow, 1.8 mm in diameter, with slightly undulate margins. On steamed potato, the growth is profuse, deep yellow and glistening. In broth the growth is turbid with a yellow pellicle and heavy sediment. Gelatin is liquefied 6—7 days after inoculation. Starch is not hydrolysed. Neither H<sub>2</sub>S nor indol are formed. Nitrates are reduced to nitrites. Milk is coagulated with separation and partial digestion within 20—30 days. Good growth is produced in Uschinsky's solution with formation of a yellow pellicle and heavy sediment. Acetylmethylcarbinol is produced. There is good growth at 3 and 5% NaCl and medium growth at 7%. Acid reactions but no gas are produced on glucose, lactose, sucrose, maltose, mannitol, mannose, glycerol, xylose, salicin, and sorbitol.

Of all the species listed in the genus *Erwinia*, *E. lathyri* (Manns and Taubenhaus) Holland<sup>2</sup> seems to be the closest. This organism is stated to be pathogenic for sweet pea and other legumes, but it is considered by many to be a saprophyte<sup>2</sup>. It differs, however, from the banana organism in the following main points: it forms indol, it does not reduce nitrates to nitrites and its sensitivity to NaCl is greater<sup>2</sup>. There is no doubt, however, that the banana organism should be



considered a wound pathogen. Taking into consideration the above differences, it is finally suggested that the banana organism be identified as a pathogenic variety of *E. lathyri*.

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## BOOK REVIEW

J.T. BONNER: *Morphogenesis: An Essay on Development*. Princeton University Press, Princeton, N.Y. 1952. 296 pp. \$ 5.00.

This essay is not just a fireside chat, but easy (and I must say fascinating) reading for the biologist acquainted with most of the facts which are presented here from a general and peculiar point of view. The main chapter deals with "Size and Pattern", and in it the author stresses that size alone is an important morphogenetic factor, as aquatic animals of less than 1 mm diameter, e.g., may dispense with respiratory and circulatory systems, while increase in size leads to differentiation in structure. In "Physics and Chemistry in Development" the properties of solid and liquid crystals are dealt with, but the morphogenetic problem is immediately posed: though a sponge spicule may consist of a single calcite crystal, its external form cannot be explained according to the position of the crystal axes, but is moulded by the surrounding tissues of the body.

In the chapter "Patterns of Growth", the more formalistic theories of relative and heterogeneous growth as well as the Cartesian transformations are discussed (almost without mathematics!). Bone structure is mentioned in connection with the directed growth of fibroblasts on stretched plasma media (P.Weiss), and in another context an interesting similar case of growth in myxobacteria on agar is discussed (p. 169). Several types of meristematic growth are found to be alike in plants, protozoa and hydroids, though the tissues involved are entirely different. The action of growth hormones in plants and animals is compared, as well as genetically based differences in growth rates.

The author, being essentially a mycologist, draws most of his examples for "Patterns of Morphogenetic Movements" from mycomycetes, myxobacteria and algae, which is as well, as these groups show relatively less complicated conditions than higher animals. But the well analysed morphogenetic movements of the amphibian gastrula and pigment patterns are not forgotten. "Polarity and Symmetry" chiefly evaluates the influence of

external factors and inherent gradients on the form of plants and animals. To indicate the broadness of the author's aspects, this chapter also includes patterns of animal associations as in schools of fish and flocks of birds during flight, etc., but: "the moral of this should be, that symmetry, or polarity, is merely a geometrical configuration, and there is nothing to exclude the idea, that the basis of this polarity during development might be vastly different in different forms..."

"Patterns of Differentiation" lays stress chiefly on the question how developmental patterns are localized in the plasma, nucleus or chromosomes, and how the reactive tissues respond to these stimuli.

In a final "Analysis of Development", three different groups of processes are described which are known to interact during morphogenesis: morphogenetic movements, growth and differentiation. They are influenced by "limiting factors", external (as food supply, mechanical forces, etc.) and internal (molecular orientation, hormones, inductors and gene products), but the action of each of these factors cannot be evaluated separately, but only in its context with all the other components, which makes the analysis of development extremely complicated.

The quest for a micro-theory of development is a philosophical as well as a scientific one, and, if Dr. Bonner has not solved the problem of morphogenesis (if somebody did, he could probably synthesize a living organism), he has at least succeeded in showing that, though individual methods of development differ, there are principles involved which appear to be universal. He presents precisely our current state of knowledge (or ignorance) regarding these principles and in many cases he states clearly what may be considered a well founded theory and what pure speculation.

An interesting and highly stimulating book, giving much food for thought.

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## NEWS AND VIEWS

### Israel Genetic Circle

The Founding Meeting of the Israel Genetic Circle, held on December 11, 1955, at the Department of Experimental Biology of the Weizmann Institute of Science, Rehovot, was devoted to the

research in genetics being carried out in this department of the Weizmann Institute.

A committee of two was elected: Dr. Leo Sachs of the Weizmann Institute and Dr. Naom Feinbrun of the Hebrew University of Jerusalem. Meetings will be held at regular intervals.



## NOTICE TO CONTRIBUTORS

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### MANUSCRIPT

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The following notation should be used:

Internal energy	$U$	Work function	$A$
Enthalpy	$H$	Gibbs' function	$G$
Entropy	$S$	Chemical potential	$\mu$

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Decimal division is indicated by use of a full stop on the line, e.g., 1.000 (one, accurate to the third place). Division of thousands is made by use of a comma, e.g., 1,000 (one thousand). Multiplication is indicated by a full stop centrally placed, e.g.  $8 \cdot 10^{12}$ .

#### Abbreviations

Titles of journals should be abbreviated according to the *World List of Scientific Periodicals*.

Units are used in the abbreviated form, in the singular, and are not followed by a full stop (only in. is followed by a full stop). The following is a list of the more common symbols: mm cm m km cm<sup>3</sup> m<sup>3</sup> g mg kg sec min hr °K °C.

#### Summary

Every paper must be accompanied by a brief but comprehensive summary. Although the length of the summary is left to the discretion of the author, 3% of the total length of the paper is suggested.

#### References

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